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IFW AF/1641

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOARD OF PATENT APPEALS AND INTERFERENCES

Attorney Docket No. 071007/0137

In re application of:

Group Art Unit: 1641

Serial No.: 08/886,044

Examiner: S. Devi

Filing Date: June 30, 1997

For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

ADDITIONAL EVIDENCE SUBMITTED MPEP 1207

U.S. Patent and Trademark Office
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Arlington, VA 22202

Sir:

This communication is responsive to the Notice of Non-Compliance dated April 29, 2004, concerning the above-referenced patent application.

The examiner notes that the new affidavit of Dr. Cross was provided for the first time with Appellants' Brief, and must be submitted in a paper separate from the Brief. Appellants now separately forward Dr. Cross's declaration and relevant comments regarding this declaration under MPEP 1207. This declaration is appended to this response as Exhibit A.

Dr. Cross's declaration shows that applicants have obtained early results with their vaccine in human volunteers. These results were not submitted sooner because they just recently became available. The results are reported in a further declaration by Dr. Cross and are in agreement with results obtained with the mouse model. Active immunization with the vaccine according to the present invention produced a 2- to 5-fold increase in the 24 volunteers following a 3 dose regimen. Moreover, all three doses of the vaccine were well-tolerated by all volunteers, as was a subsequent booster given at 8 months to 6 of the

volunteers. This is in distinct contrast to the "safe" heat-killed vaccine of Schwartzer, which produced pain, tenderness, induration, and erythema beginning within 6 hours of administration, as well as more severe reactions, including myalgia, low-grade fever, chills, sweats, abdominal cramps, nausea, diarrhea, and back pain (see above).

Appellants respectfully request entry of Dr. Cross's declaration into the record for the Appeal.

Respectfully submitted,

28 May 2004
Date

S. A. Bent
Stephen A. Bent
Reg. No. 29,768

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 071007/0137

In re application of:
BHATTACHARJEE *et al.*

Group Art Unit: 1641

Serial No.: 08/886,044

Examiner: S. Devi

Filing Date: June 30, 1997

For: *VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS*

DECLARATION UNDER 37 CFR §1.132

Assistant Commissioner of Patents
Washington, D.C.

Sir:

I, Alan S. Cross, M.D., declare and say as follows:

1. I am the Alan S. Cross shown as co-inventor on the captioned patent application. I am experienced in the field of vaccines directed against bacterial infections. My curriculum vitae is appended to my prior declaration.

2. An initial human study with a vaccine according to the present invention has recently been completed. Twenty four human volunteers were divided into three groups of eight volunteers each. The three groups were inoculated with J5 *E. coli* LPS/*N. meningitidis* OMP vaccine containing 5, 10 or 25 μg, respectively, based on the weight of the J5 LPS. The subjects in each group were inoculated on days zero, 14 and 28. Six of the subjects received a booster dose at ten months.

3. The vaccine was well-tolerated by all individuals, and increased IgG antibody titer to J5 LPS by 2- to 5-fold over pre-immunization levels in 15/24 volunteers.

Serial No.: 08/886,044

****

I hereby declare that all the statements made herein of my known knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 24, 2000
Date

Alan S. Cross

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOARD OF PATENT APPEALS AND INTERFERENCES

In re application of:

Group Art Unit: 1641

Serial No.: 08/886,044

Examiner: S. Devi

Filing Date: June 30, 1997

For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

THIRD REVISED BRIEF ON APPEAL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOARD OF PATENT APPEALS AND INTERFERENCES

Attorney Docket No. 071007/0137

In re application of: Group Art Unit: 1641

Serial No.: 08/886,044 Examiner: S. Devi

Filing Date: June 30, 1997

For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

APPELLANTS' THIRD REVISED BRIEF UNDER 37 CFR §1.192

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Arlington, VA 22202

Sir:

This brief is being filed in response to the further Notification of Non-Compliance with 37 CFR 1.192(c) mailed April 29, 2004. The fees required under 37 CFR §1.17(f) were included in our Check No. 4716 filed with the initial Brief on July 24, 2000. Please charge any fee deficiency or credit any overpayment to Deposit Account 19-0741.

This brief is transmitted in triplicate in conformance with 37 CFR §1.192(a).

REAL PARTY IN INTEREST

The real party in interest in this case is the Government of the United States, as represented by the Secretary of the Army.

1. ***RELATED APPEALS AND INTERFERENCES***

There are no appeals or interferences, which will directly affect, be affected by, or have a bearing on, the Board's decision in this case.

2. *STATUS OF CLAIMS*

Pending: Claims 1-3, 5-8, and 15-17

Canceled: Claims 4, 9-14, and 18-20

Rejected: Claims 1-3, 5-8 and 15-17

Appealed: Claims 1-3, 5-8, and 15-17

3. *STATUS OF AMENDMENTS*

Two responses were submitted following final rejection. The first response was submitted on January 19, 2000, and cancelled claims 19 and 20. An Advisory Action dated March 15, 2000, indicated that the examiner had considered this response, and that for purpose of appeals, claims 1-3, 5-8 and 15-17 were pending and rejected. A second response was submitted on April 18, 2000, and canceled claims 12-14, 19 and 20. An Advisory Action dated June 1, 2000, indicated that the examiner had considered this response as well, and that it had been entered into the record for appeal. The claim status above reflects these amendments.

4. *BACKGROUND AND SUMMARY OF THE INVENTION*

The present invention relates to a vaccine effective against infections with Gram-negative bacteria and lipopolysaccharide ("LPS")-mediated pathology induced by Gram-negative bacterial infections. More particularly, it relates to a non-covalent, polyvalent complex vaccine containing purified *E. coli* LPS endotoxin and purified *N. meningitidis* outer membrane protein, which vaccine produces, in an actively immunized subject, an immune response against Gram-negative bacterial infection and the pathology caused by the LPS endotoxin.[1]

Infections by Gram-negative bacteria and consequent septic shock are leading causes of death among hospitalized patients. It is estimated that Gram-negative sepsis has an incidence of 70,000 to 300,000 cases per year in the United States. McCabe *et al., Am. J. of Med.* 68: 344 (1980). While attempts have been made to produce vaccines that will produce anti-endotoxin antibodies, and thereby protect against septic shock, results have been

[1] Specification at page 1, lines 8-17.

disappointing. For reviews, see Cross *et al., J. Endotox. Res.* 3: 57 (1994) and Greisman and Johnston, *J. Endotox. Res.* 4: 123 (1997).[2]

Because of an unmet need of long-standing for a vaccine effective against Gram-negative bacterial infections, the present inventors have devised a novel vaccine which allows for both active and passive immunization against Gram-negative bacterial infections. The present invention provides a vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology by the production of an antibody, comprising a non-covalent complex between (i) purified, detoxified LPS endotoxin derived from *E. coli* J5 strain and (ii) a purified outer membrane protein (OMP) derived from *N. meningitidis.*[3] The *E. coli* J5 strain is a so-called rough mutant strain of *E. coli* which produces LPS that lacks O-side chains.[4] The present invention also provides a method of actively immunizing a subject against infection by heterologous Gram-negative bacteria and LPS endotoxin-induced pathology, comprising administering to said subject an effective amount of a vaccine according to the invention.[5]

5. ISSUES

The sole issue on appeal in this case is whether claims 1-3, 5-8 and 15-17 would have been obvious based on Zollinger *et al.* (U.S. 4,707,543) in view of Ziegler *et al.* (*New Engl. J. Med.* 1982) or Myers *et al.* (U.S. 4,912,094) and Munford *et al.* (U.S. 4,929,604).

6. GROUPS OF CLAIMS

For purposes of this appeal, the claims do not all stand or fall together, but will be argued separately according to the following groups:

Group I Claims 1-3, 5, and 15-17

---

[2] Specification at page 1 line 23 to page 2, line 1; Greisman and Johnston (1997) was provided and first discussed in response dated January 14, 1999.

[3] See claim 1, appended.

[4] See, for example, Ziegler or Calandra, of record. Both Calandra's work and Ziegler's work also are discussed in the specification on page 2, lines 1-9 and 19-21.

[5] See claim 6, appended.

Group II Claims 6-8 (see pages 25-26 of the Brief)

7. SUMMARY OF THE ARGUMENT

As elaborated below, the examiner has misapplied the primary reference, because it is not true that "Zollinger *et al.* teach the use of detoxified LPS from *Escherichia coli*, non-covalently complexed with OMP of group B *Neisseria meningitidis*, as a vaccine against infection." In the first instance, substitution of J5 LPS for the generic LPS in Zollinger must be based on an assumption that J5 will behave equivalently in terms of solubilizing OMP, an assumption that is not supported by the teaching in Zollinger. In addition, the underlying basis for the rejection based on Zollinger, that any combination of polysaccharide and OMP will produce equivalent results in a vaccine, has been conclusively rebutted by evidence in the record. And even if the essence of Zollinger were to combine LPS from *E. coli* and OMP from *N. meningitidis*, one skilled in the art would not have been motivated, by "the expected benefit of using an immunogen that elicits protective response against multiple pathogenic bacterial species," to substitute J5 LPS of Ziegler in Zollinger's complex, and there would have been "no reasonable expectation of success in using such a composition in active or passive immunizations against Gram negative bacterial sepsis." Moreover, the examiner has relied on teachings from Munford and Myers, to the effect that the structure of the R core region of LPS "is similar in most gram negative bacteria," despite applicants' demonstration that these teachings were debunked in the art when the present application was filed. The fact remains that, notwithstanding the widespread belief that J5 LPS is not an effective immunogen, applicants have succeeded in providing the key to unlock J5 LPS's latent immunogenicity.

8. ARGUMENT

The examiner's stated rejection

The claims in this case are rejected, under Section 103(a), over the combination of Zollinger in view of Ziegler or Myers and Munford. For a full expression of the basis for this rejection, it is necessary to return to the Official Action dated September 14, 1998. There the examiner urges that:

> Zollinger *et al.* teach the use of detoxified LPS obtained from *Escherichia coli*, non-covalently complexed with OMP of group B *Neisseria meningitidis* as a vaccine against infection...
>
> Ziegler *et al.* teach a purified LPS of *E. coli* J5 and its role as an effective immunogen.
>
> Myers *et al.* teach that "the core region is highly conserved among LPSs obtained from different genera of *Enterobacteriaceae* and that immunity against the core region is...protective against a wide variety of Gram negative bacterial challenges" and was "demonstrated by the work of Ziegler *et al*....
>
> Munford *et al.* teach that "the structure of the lipid A moiety is highly conserved' in the LPS of many pathogenic bacteria...that LPSs may be used as vaccines to prevent gram negative bacterial sepsis by producing antibodies to R-core regions....[and that] the structure of the R core region of LPS "is similar in most gram negative bacteria"...
>
> With this perspective, the examiner concludes that:
>
> It would have been obvious to one skilled in the art at the time the invention was made to substitute Zollinger's generic *Escherichia coli* LPS with its O-specific side chains intact, with Ziegler's or Myers' specific *E. coli* J5 LPS which is devoid of O-specific side chains, to produce the instant invention because, Zeigler *et al.* teach that O-specific side chains present in the LPS of parent *E. coli* strain 'conceals' the protective core determinants whereas *E. coli* LPS devoid of O-specific side chains has this protective core determinant exposed (and thus available for recognition by the host immune system). One skilled in the art would be motivated to produce the instant invention for the expected benefit of using an immunogen that elicits protective response against multiple pathogenic bacterial species in addition to *E. coli* (for example *S. typhimurium* and the meningococcus) because the exposed/unblocked conserved antigenic determinants that this immunogen presents to the host immune system as taught by Ziegler *et al.* or Myers *et al.* or Munford *et al.* One skilled in the art would have had a reasonable expectation of success in obtaining the vaccine of the instant invention since Ziegler's purified *E. coli* J5 LPS would be expected to function no differently than Zollinger's generic *E. coli* LPS when complexed with meningococcal outer membrane protein.

The most recent action expands on the examiner's rationale for combining OMP from *N. meningitidis* and J5 LPS from the *E. coli* J5 strain. In particular, she urges that the skilled practitioner would have been motivated "to substitute Zollinger's generic *E. coli* LPS with Zeigler's specific J5 LPS to produce the instant composition and vaccine for the expected benefit of economically and advantageously immunizing a subject against heterologous Gram negative bacterial sepsis with a single, all-in-one composition, with a reasonable expectation of success

in using such a composition in active or passive immunizations against Gram negative bacterial sepsis."

It is not true that "Zollinger et al. teach the use of detoxified LPS from Escherichia coli, non-covalently complexed with OMP of group B Neisseria meningitidis, as a vaccine against infection."

Zollinger discloses a "process for preparing a detoxified polysaccharide-outer membrane protein complex from bacterial envelopes. The so-obtained products which are useful against infection by the ***same*** bacteria" (abstract, emphasis added). The ***purpose*** of the polysaccharide in Zollinger, whether capsular polysaccharide or lipopolysaccharide, is to solubilize the outer membrane proteins.

Thus, Zollinger speaks of "outer membrane proteins...solubilized by the tetravalent mixture of A, C, Y, and W135 polysaccharides" (col. 2, lines 7-9). He also states that "the detoxified [lipopolysaccharide] was shown to retain its ability to bind to and ***solubilize*** outer membrane proteins" (col. 8, lines 66-68), and that "sonication is often essential to facilitate the protein-lipopolysaccharide interaction and ***solubilize*** the protein" (col. 9, lines 13-15; emphasis added in each case). For the purpose of solubilization, either detoxified lipopolysaccharide or capsular polysaccharide can be used, *i.e.* the teaching of Zollinger is that all lipopolysaccharides and capsular polysaccharides are equivalent. Zollinger teaches that the process in question is applicable generally to the preparation of detoxified polysaccharide-protein complexes derived from gram-negative bacteria, preferably *Neisseria meningitidis* group B, *Haemophilius influenze* type b, *N. gonorrhoeae*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

From the foregoing, it is apparent that the examiner has applied Zollinger in a selective manner, picking OMP from one species (*N. meningitidis*) and detoxified LPS from another (*E. coli*). ***This mixing and matching is not suggested by Zollinger, however.***

Indeed, Zollinger's only specific examples, one with capsular polysaccharide and another with detoxified lipopolysaccharide, entail the use of OMP from the ***same species*** as the polysaccharide. In his Example 1, outer membrane protein from *N. meningitidis* is complexed with capsular polysaccharides from serogroups A, C, Y, and W-135 of *N. meningitidis*. In Example 3, outer membrane protein from *N. meningitidis* is complexed with detoxified lipopolysaccharide from a serogroup B case of *N. meningitidis*.

The use of outer membrane protein and polysaccharide from the ***same species*** is consistent with another Zollinger teaching, that the vaccine is directed against the same species from which the OMP is obtained. If the polysaccharide is to fulfill any purpose in addition to the solubilization function taught by Zollinger, the clear indication in the reference is that it serve to strengthen the antigenic response to the OMP. This would best be achieved by using polysaccharide from the same species as the OMP.

Accordingly, the allegation that Zollinger teaches a combination of OMP from *N. meningitidis* and LPS from *E. coli* is a hindsight reconstruction of the art and, hence, should be withdrawn. Such an *N. meningitidis/E. coli* combination not only falls outside the ambit of Zollinger's teachings but also is contrary to Zollinger's express purpose.

Substitution of J5 LPS for the generic LPS in Zollinger must be based on an assumption that J5 will behave equivalently in terms of solubilizing OMP, an assumption that is not supported by the teaching in Zollinger.

Since Zollinger does not specifically teach the use of endotoxin derived from J5 mutant, the basis for the present rejection must be that it would have been obvious to substitute endotoxin from this mutant for capsular polysaccharides or for lipopolysaccharide purified from a serogroup B case strain because equivalent results would be achieved, *i.e.*, ***that the J5 LPS would be equally effective in solubilizing the outer membrane protein***. The premise on which the rejection is based must be that J5 lipopolysaccharide would be expected to behave equivalently in combination with outer membrane protein ***in terms of the ability to solubilize outer membrane protein, since that is the purpose of the LPS in Zollinger.***

Would LPS without O-chains be expected to solubilize outer membrane protein as effectively as LPS with O-chains? Zollinger did not use LPS without side chains, and so provides no direct guidance on this issue. However, Zollinger does specifically comment at the bottom of column 8 that "the detoxified product was shown to ***retain its ability to bind to and solubilize outer membrane proteins***" (emphasis added). That is, Zollinger felt it necessary to comment on whether a modification of LPS to remove part of it, *i.e.*, the Lipid A moiety, would affect its ability to achieve the stated purpose for the LPS in the Zollinger, namely the ability to bind to and solubilize outer membrane protein. This suggests uncertainty over whether an LPS molecule modified to remove Lipid A would retain the necessary solubilizing properties. A skilled artisan might doubt, as well, the ability of

LPS without the O-chains effectively to solubilize outer membrane protein, thereby undermining the alleged case of obviousness.

The underlying basis for the rejection based on Zollinger, that any combination of polysaccharide and OMP will produce equivalent results in a vaccine, has been conclusively rebutted by evidence in the record.

The examiner urges in her rejection that "Ziegler's purified *E. coli* J5 LPS would be expected to function ***no differently*** than Zollinger's generic *E. coli* LPS when complexed with meningococcal outer membrane protein" (emphasis added, citation above). Thus, the examiner urges equivalence as the basis for the substitution of J5 LPS for the lipopolysaccharide or capsular polysaccharide disclosed in Ziegler or Myers.

There is evidence in the record, however, that J5 LPS functions ***entirely differently*** than "Zollinger's generic *E. coli* LPS" when complexed with OMP. Forwarded with applicants' response submitted June 19, 1998, was a declaration by Dr. Cross, one of the co-inventors of the present claims.[6] In the June 19th declaration, Dr. Cross discusses studies which compare combinations of OMP derived from *N. meningitidis* and purified, detoxified LPS endotoxin derived from *E. coli* strain J5 and combinations of OMP with purified, detoxified LPS endotoxins from other strains of *E. coli*.

The comparison in the June 19th declaration is a comparison to more than that which is shown in any concrete example in Zollinger. As noted above, the only concrete examples in Zollinger relate to combinations of OMP and LPS which both are from *N. meningitidis*. Thus, in Example 1, outer membrane protein from *N. meningitidis* is complexed with capsular polysaccharides from serogroups A, C, Y, and W-135 of *N. meningitidis*, while in Example 3, outer membrane protein from *N. meningitidis* is complexed with detoxified lipopolysaccharide from a serogroup B case of *N. meningitidis*. Applicants compare to a combination of OMP from *N. meningitidis* with LPS from *E. coli*, the combination alleged by the examiner to be generically encompassed by Zollinger. In other words, the comparison performed by applicants relates to the examiner's hindsight interpretation of Zollinger.

Experiments described in paragraphs 2-5 of the June 19th declaration examined the efficacy of active immunization with four different vaccines. A first group of mice was immunized only with *N. meningitidis* OMP, a second with *Brucella* LPS

[6] For convenience, copies of the three Cross declarations are appended in Exhibit 1.

complexed to *N. meningitidis* OMP, a third with J5 LPS complexed to OMP, and a fourth with EC018 LPS complexed to OMP. Vaccination with J5-OMP led to 90% survival, 50% greater protection than vaccination with EC018-OMP, a complex of OMP with another strain of *E. coli*. The results in the Cross declaration therefore show that combinations of OMP derived from *N. meningitidis* and purified, detoxified ***LPS endotoxin derived from E. coli strain J5*** provide unexpectedly superior protection against Gram-negative sepsis as compared to combinations of OMP with purified, detoxified ***LPS endotoxins from other strains of E. coli***. This is particularly surprising in view of the fact that J5-OMP vaccine was providing protection against a heterologous strain (EC018) whereas EC018-OMP was providing protection against the same strain.

These results could not have been predicted based on Zollinger, which teaches that capsular polysaccharides and wild-type lipopolysaccharides behave ***equivalently*** in combination with an outer membrane protein for the purpose of solubilization as disclosed in Zollinger. This evidence conclusively rebuts the examiner's allegation that "Ziegler's purified *E. coli* J5 LPS would be expected to function no differently than Zollinger's generic *E. coli* LPS when complexed with meningococcal outer membrane protein."

> ***One skilled in the art would not have been motivated, by "the expected benefit of using an immunogen that elicits protective response against multiple pathogenic bacterial species," to substitute J5 LPS of Ziegler in Zollinger's complex, and there would have been "no reasonable expectation of success in using such a composition in active or passive immunizations against Gram negative bacterial sepsis."***

It is the PTO's burden to find, within the cited art, evidence of motivation for the skilled artisan to have complexed LPS from the J5 strain, instead of detoxified LPS or capsular polysaccharide from other strains, with an outer membrane protein from N. meningitidis. Casting about for such evidence, the examiner argues that the J5 LPS would serve "as an immunogen to treat sepsis caused by multiple gram negative bacterial pathogens" based on Ziegler *et al.*

At an interview on February 14, 2000, however, Dr. Cross explained that all previous attempts to immunize or otherwise protect individuals against LPS endotoxin-mediated pathology had been unsuccessful. There is perhaps no better substantiation of this statement than a review article by Greisman and Johnston, published in 1997 (of record). Entitled "Evidence against the hypothesis that antibodies to the inner core of

lipopolysaccharides in antisera raised by immunization with enterobacterial deep-rough mutants confer broad-spectrum protection during Gram-negative bacterial sepsis," the Greisman/Johnston article is much more recent than any of the art cited by the examiner. Moreover, Dr. Greisman is a well-respected expert in this field and has received Honorary Lifetime Membership in the International Endotoxin Society, the highest award bestowed by that organization upon investigators involved in endotoxin research.

Included among the articles reviewed by Dr. Greisman is Ziegler *et al.*, *New England J. Med.* (1982), the only reference, presently recited, that relates to LPS from J5 mutants. The Greisman article also considers many of the prior-art attempts to achieve protection with antisera to LPS from J5 mutants. As to each and every study implicating broad-spectrum protection by rough-mutant antisera, Dr. Greisman concluded that defects, in design or methodology, had engendered inconsistent results, and that antisera to the J5 chemotype "do not appear capable of providing broad-spectrum protection."

The Ziegler article, cited by the examiner against the present claims, is discussed in the last paragraph on page 126 of Greisman *et al.* Greisman notes Ziegler's report of broad-spectrum protection with antisera to J5 rough mutants. In view of Ziegler's results, Dr. Greisman reevaluated the putative anti-LPS effect of rough mutant antisera, but first screened the antisera to preclude polyclonal increments in O-specific antibodies to the challenge LPS, and used pre-immune sera from the respective donors as controls.[7] When these steps were taken, Dr. Greisman found that rabbit antisera to J5 mutant, which possessed titers of antibody to the respective LPS core determinants comparable to or higher than those used by investigators who reported broad-spectrum protection against LPS, nevertheless failed to protect mice against lethality produced by LPS from heterologous smooth enterobacteria or even from the homologous smooth parental strain. According to Dr. Greisman, these results "failed to support the hypothesis that antisera to J5 and R595 are capable of effective broad-spectrum neutralization of the lethal activity of S-form LPS." *Id.* at page 127.

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[7] Also of record are the following articles by Dr. Greisman, relating to this reevaluation:

"Failure of Antisera to J5 and R595 Rough Mutants to Reduce Endotoxemic Lethality," *J. Inf. Dis.* 157:54-63 (1988), and

Dr. Greisman's conclusion regarding Ziegler's study was shared by others in the field. Indeed, Ziegler herself was unable to identify antibodies as a basis for the protection observed ("we could not relate protection to the J5 antibody titer, regardless of the immune status of the donor" – page 1228, last paragraph). Zanetti *et al.* (of record) stated that, "as was noted in the report by Ziegler *et al.*, protection was related to immune plasma, not to specific levels of antibody to core LPS in a given plasma" (first paragraph on page 988) and, "as already noted, in both successful clinical studies with *E. coli* J5 antiserum, the protection remained of unclear origin because outcome could not be convincingly correlated with the level of antibodies to the core LPS of *E. coli* J5...the protection afforded by *E. coli* J5 antiserum could not be attributable to antibodies to the LPS of *E. coli* J5" (second full paragraph on page 988). Similarly, Glauser *et al.* (of record) noted that "a favorable outcome could not be correlated with antibody titers in either of the two clinical studies done with human polyclonal antisera to J5...the mechanisms of protection by antisera to J5 remain unknown" (second full paragraph on page S208). Baumgartner opined that "the successful studies did not discover the factor responsible for the postulated crossprotection in J5 antiserum, because the protection could not be attributed to anti-J5 LPS, anti-Re LPS, or anti-lipid A antibodies" (top of page 923).

In subsequent clinical trials, use of an anti-J5 LPS monoclonal antibody, rather than polyclonal antiserum from donors, fared no better in providing protection. Results of these trials were appended as Exhibit 2 in applicants' response filed April 18, 2000.

In the first of these, a trial by Ziegler *et al.* which was reported in the *New England Journal of Medicine* in 1991, the mortality rate among all patients with sepsis was 43 percent among recipients of placebo and 39 percent among those given HA-1A. Ziegler concluded that this result is similar to that obtained in their earlier trial with polyclonal antiserum. Two trials with HA-1A that were conducted by other researchers, however, produced different results. A trial reported by McCloskey *et al.* in 1994 in the *Annals of Internal Medicine* concluded that "HA-1A was not effective in reducing the 14-day mortality rate in patients with gram-negative bacteremia and septic shock." A trial in children with meningococcal septic shock, as reported by Derks *et al.* in a 1999 issue of *Clinical Infectious Diseases*, concluded that "no significant benefit of HA-1A could be demonstrated."

"Experimental Gram-Negative Bacterial Sepsis: Reevaluation of the Ability of Rough

Dr. Greisman reviews results in five other clinical trials, in a declaration appended as Exhibit 4 to applicants' April 18th response. In his declaration, Dr. Greisman attests to failures of five clinical trials to show broad-spectrum protection during Gram-negative bacterial sepsis.[8] All of these trials were subsequent to Ziegler's clinical study of polyclonal antiserum, reported in 1982 and cited by the examiner.

In the first negative trial, performed by Braude and Ziegler's group, pre- and post-immune J5 antisera were given prophylactically to patients with neutropenia. The results, however, evidenced no differences in the rates of Gram-negative bacteremia, febrile episodes or mortality. In the second clinical study, a gamma globulin fraction from donors with elevated antibody titers to J5 LPS proved ineffective. In a third trial, an IgG fraction from sera of volunteers immunized with the J5 mutant provided no more protection against mortality than the IgG fraction from standard plasma pools, and did not reduce the number systemic complications of shock and did not delay the occurrence of death from systemic shock. A fourth clinical trial, in which infusions of human immunoglobulin preparations selected for their high content of IgG to R595 LPS, failed to show any greater protection against subsequent Gram-negative bacterial infections or their systemic complications in patients at high risk after major surgical procedures than was achieved with comparable immunoglobulin preparations containing on average 7-fold lower amounts of anti-R595 IgG. Finally, in a fifth clinical trial, 73 children with severe infectious purpura, the majority secondary to *N. meningitidis*, received J5 immune or pre-immune plasma. The anti-J5 plasma did not affect the clinical course, or the rate of decrease of TNFα or IL-6 or mortality.

In both his declaration and his review article, Dr. Greisman documents other attempts to demonstrate the protective capacity of J5 antisera in the laboratory. Many of the articles reviewed in Greisman *et al.* are the very ones listed by the examiner as showing the state of the art, including work by McCabe, Dunn, Young, Braude, Davis, Cryz, Di Padova, Nelles, Lugowski, and Salles. These articles have not been cited against the claims, and applicants will not discuss the flaws in each of them; to do so would risk obscuring the forest for the trees. Rather, applicants rely on the considerable expertise of Dr. Greisman in thoroughly reviewing the state of the art in this field, *circa* 1997. Dr. Greisman's review

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Mutant Antisera to Protect Mice," *Proc. Soc. Exp. Biol. Med.* 158:482-490 (1978).

[8] Copies of articles relating to each of these five clinical trials were appended as Exhibit 5 to the April 18th response.

reveals the flaws in each of these studies, which led to divergent results, and after his thorough review Dr. Greisman still believes that an anti-endotoxin vaccine for effective broad spectrum therapy of sepsis is unlikely to be developed.

Indeed, Dr. Greisman is not alone in his opinion. In March Dr. Cross attended a worldwide conference on sepsis in Europe. Over 1,000 papers were presented, yet only two dealt with antibodies against endotoxin, one by Dr. Cross and one on the WN1 monoclonal of Sandoz. Dr. Dunn, whose work in hyperimmunizing horses was included in the "state of the art" referenced by the examiner, was present. He was, however, silent on the possibility of an LPS vaccine.

In short, Dr. Greisman's article and declaration, as well as the other information discussed in this section effectively rebut the contention that a skilled artisan would reasonably have expected J5 LPS to act as an effective immunogen in a vaccine preparation to elicit a protective response against multiple pathogenic bacterial species, and more particularly against sepsis. Since antibody induced in response to J5 LPS alone does not provide effective protection against sepsis, the argument that it would have been obvious to complex LPS from a J5 strain with OMP from *N. meningitidis* instead of LPS from *N. meningitidis* "as an immunogen to treat sepsis" is totally without basis.

In an Advisory Action dated June 1, 2000, the examiner suggests that Dr. Greisman is a lone voice crying in the wilderness -- that he "fail[ed] to cite and/or discuss a plethora of positive studies, published in the art prior to the filing of the instant application."[9] There are others, however, who echo Greisman's sentiments on this subject. Like Greisman, a highly respected group in Switzerland led by Baumgartner and Glausner also has questioned the strength of the data presented in positive studies. An article by Baumgartner (1991), of record, reviews many unsuccessful clinical trials. Of the few successful trials reviewed, "the protection could not be attributed to anti-J5 LPS, anti-Re LPS or anti-lipid A antibodies" (page 923). And as recently as 1997, Zanetti and Glauser conclude that the "failure of these trials concluded three decades of research on anti-endotoxin approaches..."[10] And even an article on which Munford is a coauthor states that "proof that therapies

[9] Advisory Action at page 11.

[10] A copy of Glauser and Zanetti was appended to the Additional Evidence Submitted under MPEP 1207 filed January 8, 2004.

specifically targeting endotoxins work in human septic shock is still lacking."[11] So Greisman is not alone in his pessimistic views relating to prevention and treatment of sepsis.

One of the "positive" trials, alleged by the examiner to have been ignored by Greisman, is Cryz *et al.* The examiner argues that:

> Cryz *et al.* (*Eur. J. Clin. Microbiol.* 4:180-185, 1985) demonstrate, by active immunization, that an O-polysaccharide-deficient lipopolysaccharide derived from *E. coli* J5 LPS (administered in the absence of a strong adjuvant such as group B meningococcal OMP) is immunogenic in mice....Mice immunized with J5 LPS alone showed 70% protection against challenge with a heterologous Gram negative bacterium, such as *Pseudomonas aeruginosa* E576....this study provided the biological evidence of cross-reactivity and cross-protection, against a heterologous Gram negative pathogen, afforded by an unconjugated or non-complexed purified J5 LPS.[12]

Cryz *et al.* clearly state that anti-core antisera derived from J5 afforded substantial protection against only one of five *Pseudomonas* challenge strains. They conclude in their abstract that "attempts to demonstrate cross-serotype protection using O-antigen deficient and core deficient *Pseudomonas aeruginosa* lipopolysaccharide antigens was, for the most part, unsuccessful" (emphasis added). Amazingly, the examiner cites the data only on that one strain of *Pseudomonas* (shown in Table 2, p. 182), totally ignores the lack of protection on the other four, and completely ignores the authors' own conclusions. This is not only unfair to the authors, but evidences an inability on the part of the examiner to accurately characterize the disclosure of art that she has cited.

Moreover, Greisman did not ignore Cryz -- it is discussed on page 127 of the Greisman article. There it is noted that "Cryz *et al.* demonstrated that murine antisera to J5 LPA failed to cross-react in an ELISA with S-form LPS from three wild-type strains of *P. aeruginosa*."

Other "positive" studies listed by the examiner on pages 11-18 of the Advisory Action as being ignored by Greisman were, in fact, dealt with by Greisman. Thus, Greisman notes on page 126 that Wickstrom *et al.* (article K in the Action), demonstrated that although bovine J5 antiserum was more protective than saline against *E. coli* sepsis in calves, 'normal

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[11] Morrison *et al.*, *ASM News*, 60:479 (1994), appended to the Additional Evidence Submitted under MPEP 1207 filed January 8, 2004..

[12] Advisory Action at page 11-12.

serum was almost as helpful as immune [j5] serum'." The studies of Schwartzer *et al.* (article L in the Action) are characterized among references 35-52 on page 126 of Greisman as showing an "inability to demonstrate significant protection after either active immunization with rough-mutant bacterial vaccines of passive immunization with the resultant antisera." The examiner characterized Schwartzer as teaching immunization with a "safe, heat-killed *E. coli* J5 vaccine." This is, at best, an overly favorable characterization of Schwartzer's vaccine. Indeed, of 16 vaccinees, only 9 returned for a second inoculation. The other 7 refused a second inoculation, based on their adverse reactions after this first injection, which included pain, tenderness, induration, and erythema beginning within 6 hours of administration, as well as more severe reactions, including myalgia, low-grade fever, chills, sweats, abdominal cramps, nausea, diarrhea, and back pain. Generally, a vaccine with such a high incidence of such severe side effects would not be characterized as "safe." The examiner states that "in 50% of the vaccinees, a four-fold or greater response in anti-J5 LPS antibody response was mounted." She carefully ignores, however, Schwartzer's report that the response was transient and not increased by revaccination.

Applicants also have provided evidence that teachings from Munford and Myers, to the effect that the structure of the R core region of LPS "is similar in most gram negative bacteria," were debunked in the art when the present application was filed.

Myers is cited by the examiner as teaching that the core region is highly conserved. This fact has long been known. While there are highly conserved epitopes in the LPS core, however, others have shown that there exists a microheterogeneity in these epitopes. See, for example, Table 1 in the manuscript "Vaccines and Antibodies in the Prevention and Treatment of Sepsis" and Figure 11 of Lugowski (of record). In studies by Lugowski *et al.* (1996), in which core LPS from *E. coli* was used as a vaccine, there was no binding to *Klebsiella* (applicants' J5 LPS/OMP vaccine does bind to *Klebsiella*; see below). Moreover, there was little cross reaction between antiserum raised against the core LPS of J5 and other cores from *E. coli*, including the prototype core R3 to which J5 *E. coli* belongs! Thus, even within *E. coli*, there are significant differences between core epitopes.

In the Advisory Action dated June 1, 2000, the examiner responds that Figure 3 of Lugowski, *FEMS Immunology and Medical Microbiology* 16:21 (1996):

> convincingly demonstrates that, despite the stated structural microheterogeneity, an antiserum raised to *E. coli* R3 (J5) conjugate, in the

> absence of a strong adjuvant such as group B meningococcal OMP, cross-reacted well with the LPS of heterologous Gram negative bacteria including that of *Sh. flexneri, Citrobacter,* R2 core prototype of *E. coli, E. coli* 0111 serotype, and to some extent, also with the LPS of *Klebsiella pneumoniae, S. typhimurium* and Ra prototype of *E. coli.*[13]

Figure 3 of Lugowski shows antibody raised against the R3 core, the type of core seen in J5, but there is no way that Figure 3 shows clinically significant cross-reactivity. Figure 3 shows minimal, if any, binding to other cores of *E. coli*, and no binding to *Klebsiella* or *Salmonella*! Indeed, Lugowski concludes, on page 24, that the anti-R3 serum "shows the highest specificity among tested antisera. At low concentration it reacts only with R3 core type lipopolysaccharide and with *Citrobacter* 1487 LPS possessing closely related core type." (emphasis added). It is not understood how the examiner can possibly cite Figure 3 of Lugowski (1996) as suggesting cross-reactivity. Furthermore, "detectable" binding and the real binding indicative of a useful vaccine are quite different things. The very minimal degree of binding to E. *coli* that is disclosed in Lugowski would never prompt a skilled artisan to proceed with vaccine studies.

The examiner also notes in the Advisory Action that Lugowski (1996) was published after applicants' priority date. Appended to the Additional Evidence Submitted under MPEP 1207 filed January 8, 2004 is a paper by Munford published in 1980 in *J. Bacteriology* 144:630. The paper is entitled "Size heterogeneity of *Salmonella typhimurium* LPS in outer membranes and culture supernatant membrane fragments." Thus, even given Munford's later claims of homogeneity, he recognizes some heterogeneity. Another earlier article which mentions the heterogeneity of the core is Gibb *et al.*, *J. Infect. Dis.* 166:1051 (1992), a copy of which was appended to Additional Evidence Submitted under MPEP 1207 filed January 8, 2004.. Gibb *et al.* examined 180 clinical isolates and found that 123 had an R1 core, 14 had an R2 core, 18 had an R3 core and 25 (14%) had none of these core types. This clearly shows the present of at least four core regions, with different patterns of reactivity. In addition to these four core regions, there exists microheterogeneity within each of the core regions, as previously discussed. For example, DiPadova was published before applicants' priority date and teaches that "microheterogeneity in the core structure is due to

[13] Advisory Action at page 7.

nonstoichiometric substitutions with phosphate and ethanolamine groups."[14] All of these differences lead to a wide variation in core conformation.

In addition to the foregoing, applicants also appended to the Additional Evidence Submitted under MPEP 1207 filed January 8, 2004 an article by Ernst Rietschel, a highly respected LPS chemist. The article publishes data suggesting that it is the conformation of the LPS core, and not its linear structure, that is important in the interaction of LPS with mammalian hosts. In other words, even if cores possess only limited heterogeneity they may interact differently in the host, since even limited heterogeneity can give rise to differences in folding, and hence, conformation. Rietschel's theory supports applicants' hypothesis, *infra*, that the OMP serves to present an important conformational epitope.

DiPadova (of record) similarly suggests a conserved core region by disclosing that a monoclonal antibody binds to the 5 known cores of *E. coli* and to *Salmonella* core. DiPadova and colleagues generated their core LPS-specific monoclonal antibody by sequential immunization of animals with different LPS core structures. Even when animals were immunized with a variety of LPS core structures, however, the resulting monoclonal antibody had no activity against *Klebsiella* or *Pseudomonas*. (Applicants' J5 LPS/OMP vaccine does bind to *P. aeruginosa* and *Klebsiella*, as shown in the declaration dated January 12, 1999, that was submitted by Dr. Cross.) In the Advisory Action, the examiner attempts to rebut DiPadova along the same lines as Lugowski. Thus she notes, on page 19, that DiPadova "did show detectable binding to the LPS." It is noted, however, that the antibody bound only to some isolates of *Klebsiella* and to no isolates of *Pseudomonas*. It would not have been obvious to make "a vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology," based on this disclosure.

In a more recent editorial, published in 1992 and appended to applicants' April 18th response as Exhibit 6, Munford and colleagues state the premise on which Ziegler's trial was based, i.e., that the highly-conserved core region generates protective antibodies and is an effective immunogen, "remains unproved and unclear." In particular, they note that the results obtained in the preclinical studies of Ziegler antibody HA-1A were "substantially

[14] DiPadove at page 3863, bottom of right-hand column.

different from those more recently described." In addition, they cited information obtained under the Freedom of Information Act that "there is no experimental model in which HA-1A has consistently protected animals from endotoxic challenge." Open Meeting of the Vaccines and Related Biological Products Advisory Committee (September 4, 1991), Volume 1, Bethesda, Md.: FDA, 1991:50. Thus, subsequent to his patent filed in 1986, Munford found reason to suspect the premise that the highly conserved core region elicits effective protection.

Finally, Myers attaches significance to the core region based on the results obtained by Ziegler in her cited 1982 article, to wit: "...the core region is highly conserved among LPSs obtained from different genera of Enterobacteriaciae; immunity against the core region is therefore protective against a wide variety of gram negative bacterial challenges. This was demonstrated by the work of Ziegler *et al.*" (emphasis added). However, as explained in detail above, Ziegler's results were not due to antibodies against the core region. Ziegler herself was unable to correlate protection with J5 antibody titer, and many others of skill in the art have commented on this point. Accordingly, Myers' teaching adds nothing to that of Ziegler, based as it is on the faulty conclusion that Ziegler showed immunity against the core region.

In an attempted rebuttal of this point in the Advisory Action, the examiner states that the J5 LPS-induced antibodies in Ziegler "conferred protection against Shwartzman reactions caused by purified endotoxins from bacterial species as widely varied as *E. coli*, *Salmonella typhimurium*, and the meningococcus," citing page 1226 of Ziegler. This clever sleight of hand on the part of the examiner attempts to use a study in which antibodies were found to protect against Shwartzman reactions in animals to counteract Ziegler's failure to correlate protection to antibody titer in their human clinical study. Protection against Shwartzman reactions in an animal study, however, does not overcome the failure of Ziegler to correlate protection to antibody titer in their clinical trial.

In sum, the inferences drawn by the examiner based on teachings in Myers and Munford can be effectively rebutted. The teaching that the core region of LPS is conserved, in light of subsequent teachings highlighted above, would not have led a skilled artisan to conclude that the core region might provide the basis for protection against sepsis.

In spite of the widespread belief that J5 LPS is not an effective immunogen, applicants persisted and have succeeded in providing the key to unlock J5 LPS's latent immunogenicity

Yet applicants have succeeded where others have failed. They have done so by complexing the J5 LPS with OMP of *N. meningitidis*. This complexation with the OMP of *N. meningitidis* appears to maintain J5 LPS in a proper spatial configuration such that relevant cross-reactive epitopes in the J5 LPS core are exposed in a manner that they are not when simply conjugated to protein or given alone. A key aspect of applicants' vaccine is reflected in their demonstration that LPS of *E. coli* J5 (Rc chemotype) - the highly conserved core of endotoxin - can produce antibodies that provide protection against the biologic activities of heterologous LPS.

Proof that a vaccine of the present invention indeed is effective, in improving the outcome following a subsequent challenge with heterologous bacteria, is manifest in the data provided in Dr. Cross's declaration of January 12, 1999 (appended in Exhibit 1). That is, the Cross declaration documents studies of challenge with virulent strains of heterologous bacteria following active immunization with J5 LPS/OMP.

As described in the protocol appended to the declaration, rats rendered neutropenic with cyclophosphamide were immunized, either with de-O-acylated J5 LPS ("dLPS") complexed to OMP or with saline, in a 3-dose regimen prior to challenge with the heterologous bacteria. Levels of antibody titer for rats immunized with the J5 dLPS/OMP exceeded a target level of 800 ELISA units/ml of antibody, a level previously shown to be protective in passive protection experiments. Following immunization, the rats were challenged with either *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*, in a dose which exceeded LD90 for this experimental model in previous studies.

The results showed that active immunization with J5 dLPS/OMP vaccine produced a prompt and sustained anti-core glycolipid antibody level that was generally in 100-fold excess of pre-immunization baseline levels. Twenty-four hours after bacteremia, antibody levels decreased, but then rapidly recovered to, and remained at, pre-infection levels. Active immunization with J5 LPS/OMP vaccine induced greater than 800 ELISA units/ml of antibody at the onset of neutropenia, nearly 4 weeks after the last dose of vaccine, and this level persisted throughout the entire period of neutropenia, for up to 80 days after the initial immunization. This is in distinct contrast to results achieved by passive immunization

with antibodies, where initial levels of 800 ELISA units/ml of antibody dropped to less than 200 ELISA units/ml of antibody by 24 hours. Thus, while levels of antibody produced in response to J5 LPS alone dropped to less than 200 ELISA units/ml of antibody by 24 hours, levels of 800 ELISA units/ml induced by immunization with LPS complexed with OMP from *N. meningitidis* are sustained for 80 days after immunization. This is clearly an unexpected result.

Immunization with J5 LPS/*N. meningitidis* OMP did not prevent either systemic infection or initiation of sepsis, but it clearly reduced the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Vaccinated animals challenged with *Pseudomonas* had an overall survival rate of 48% compared to 7% for saline treated control animals. A similar result ensued with *Klebsiella* challenge, with a 64% survival rate for vaccinated animals versus a 13% survival rate for control animals.

One particularly surprising result was the effect of the vaccine on organ colonization by the bacteria. Vaccinated animals had the same frequency and magnitude of bacteremia from the challenge strain as the control group, but had significantly lower levels of bacteria in liver and spleen than control animals.

In addition to the decreased bacterial colonization in liver and spleen, there was a significantly lower level of circulating endotoxin at the onset of fever in vaccinated animals as compared to control animals. While endotoxin levels increased in both groups at 24 hours, they were still lower than those of the control group. The lower level of circulating endotoxin may be due in part to promotion of LPS clearance from the circulation.

Antibodies produced in response to vaccination with applicants' vaccine do not appear to directly promote killing of bacteria, based on *in vitro* tests performed by applicants. They prevent neither systemic infection nor initiation of sepsis. They do, however, significantly reduce the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Dr. Cross hypothesizes that antibodies generated in response to the vaccine promote the uptake and killing of bacteria from the blood by tissue.[15]

[15] Applicant herein clarifies and corrects an earlier statement that "Antibodies produced in response to vaccination with applicants' vaccine do not appear to promote killing of bacteria, either directly or indirectly."

Inexplicably, the examiner has given short shrift to these data. In an Official Action that is 21 pages long, the examiner devotes only six sentences to the issue of applicants' declaration. On page 10 of the Action, the examiner comments that

> Applicants submit a further declaration from Dr. Cross which provides results of challenge studies with heterologous bacteria. While the information in the declaration supports that provided in the specification, it does not overcome the rejection of instant claims under 35 U.S.C. 103(a).

No support for this statement is given in context. Much later, however, in the paragraph bridging pages 14 and 15 of the Action, the examiner explains that

> The data provided with Dr. Cross's declaration shows that administration of a vaccine-derived antiserum to animals results in 48% survival of animals against challenge with *Pseudomonas* and 64% survival against challenge with *Klebsiella*. However, 52% and 36% of immunized or treated animals respectively were not "protected." The full scope of the claims is not commensurate with the scope of the enabling disclosure and undue experimentation would be required by one of ordinary skill in the art to reproducibly practice the invention as claimed. The enablement (scope) provisions of 35 U.S.C. §112, first paragraph, are not met and the claim is viewed as non-enabled with respect to its scope.

This "scope" issue, raised in connection with the declaration, is neither advanced or elaborated in the balance of the Action. The only "scope" rejection propounded in the Action relates to claim 19, which was directed to passive, not active, protection. This point was discussed at the February 14th interview, and applicants understood Examiner Housel to agree that the scope of the claims was commensurate with both the disclosure and the scope of the showing. More particularly, the undersigned explained at the interview that claim 1, applicants' broadest vaccine claim, recites "a vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology by the production of an antibody, comprising a non-covalent complex between (i) purified, detoxified LPS endotoxin derived from *E. coli* J5 strain and (ii) a purified outer membrane protein (OMP) derived from *N. meningitidis*." Thus, the broad claim specifically recites both the immunogen and the immunocarrier, as well as the fact that they form a non-covalent complex. The specification clearly describes how to make and use this vaccine, and the data in Dr. Cross's declaration uses this same vaccine and shows positive results. Based on this, it is believed that no issue of scope remains following the interview.

The examiner's comments regarding the percentage of subjects that were protected, 48% and 64%, respectively, are not understood. There are other vaccines in common use that provide significantly less than 100% protection. For example, pneumococcal immunization is routinely used, and its efficacy is generally believed to be between 60 and 70% at best. A similar degree of efficacy is the case with influenza immunization. Clearly, vaccines that provide a percentage of protection similar to that demonstrated for the present vaccine are considered to have clinical value. It is submitted that the data in the second Cross declaration are in line with that for vaccines generally, and are sufficient to rebut any allegation with respect to the enablement or obviousness of the present invention. This point also was discussed at the interview and accepted.

Applicants now have obtained early results with their vaccine in human volunteers. These results were not submitted sooner because they just recently became available. The results are reported in a further declaration by Dr. Cross, which is appended to the Additional Evidence Submitted under MPEP 1207 filed concurrently herewith, and are in agreement with results obtained with the mouse model. Active immunization with the vaccine according to the present invention produced a 2- to 5-fold increase in the 24 volunteers following a 3 dose regimen. Moreover, all three doses of the vaccine were well-tolerated by all volunteers, as was a subsequent booster given at 8 months to 6 of the volunteers. This is in distinct contrast to the "safe" heat-killed vaccine of Schwartzer, which produced pain, tenderness, induration, and erythema beginning within 6 hours of administration, as well as more severe reactions, including myalgia, low-grade fever, chills, sweats, abdominal cramps, nausea, diarrhea, and back pain.[16]

While no prima facie case of obviousness exists with respect to any of the claims, the method claims, claims 6-8, present a different issue with respect to patentability.

Zollinger clearly disclose "products which are useful as vaccines against infection by the same bacteria, and method for protecting animals against the same infection" (abstract). All of the examples of Zollinger use outer membrane protein and polysaccharide from the same species, which is consistent with Zollinger's teaching that the vaccine is directed against the same species from which the OMP is obtained. Yet the examiner maintains that it would have been obvious (1) to choose LPS from *E. coli* and OMP from *N.*

[16] *Supra.*

meningitidis in Zollinger *et al.* and then (2) to substitute J5 LPS for the generic LPS of Zollinger *et al.* to produce a vaccine.

The foregoing arguments all address the lack of any teaching in the art both to select LPS from a different species than OMP in Zollinger and to substitute J5 LPS for the generic LPS of Zollinger. But even assuming, *arguendo*, that a skilled artisan might choose, for some reason not apparent in Zollinger, to use J5 LPS to solubilize the OMP of Zollinger, that still would not have suggested the *use* of that vaccine in a method of actively immunizing a subject against infection by heterologous Gram-negative bacteria and LPS endotoxin-induced pathology, as recited in claims 6-8. Zollinger does not relate to a method of immunizing a subject against heterologous Gram-negative infection or LPS endotoxin-induced pathology. Zollinger uses polysaccharide, whether capsular polysaccharide or lipopolysaccharide, to solubilize outer membrane proteins, and uses the combination to provide protection against LPS endotoxin-induced pathology and homologous, not heterologous, infection.

To modify Zollinger's method would be contrary to the express purpose of that method as disclosed in Zollinger, to provide protection from the "same bacteria." This is in clear contravention of MPEP §2143 ("the proposed modification cannot render the prior art unsatisfactory for its intended purpose"). If the polysaccharide is to fulfill any purpose in addition to the solubilization function taught by Zollinger, the clear indication in the reference is that it serve to strengthen the antigenic response to the *same* bacteria, *N. meningitidis*. This is best achieved by using polysaccharide from *N. meningitidis*. To use a different species of LPS, and to adminster a vaccine comprisng the same in a method of providing protection against heterologous infection and LPS-induced pathology, would be contrary to the stated purpose of the primary reference, which is improper.

9. CONCLUSION

From a reading of the Official Actions in this case, one would fairly expect that an effective endotoxin vaccine must have been approved long ago. This is especially so when one considers that nearly four hundred thousand cases of sepsis a year are documented, and sepsis is the leading cause of death in intensive care units. Clearly, there is a substantial commercial market, and the search for a vaccine to prevent or alleviate the severity of sepsis has been an initial and major area of biotechnology's involvement in clinical medicine.

Yet, nearly a quarter of a century after the first publications cited by Examiner Devi, there still is no vaccine to prevent or alleviate the severity of sepsis. This highlights a basic inconsistency between what a skilled artisan would glean from the literature that is cited against applicants' claims and the reality of endotoxin vaccines. In the present case, the picking and choosing of pieces from various studies has led to an inaccurate conclusion regarding what would have been obvious in the field of endotoxin vaccines. The fact remains that no cited paper or any reasonable combination of cited papers provides the insight critical to a successful vaccine against sepsis, as presently claimed. The references lack the teaching necessary to produce a clinically useful vaccine.

For these reasons, the Board is respectfully requested to reverse the examiner and remand this application for issuance.

Respectfully submitted,

26 May 2004
Date

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APPENDIX: APPEALED CLAIMS

1. A vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology by the production of an antibody, comprising a non-covalent complex between (i) purified, detoxified LPS endotoxin derived from *E. coli* J5 strain and (ii) a purified outer membrane protein (OMP) derived from *N. meningitidis*.

2. A vaccine of claim 1, wherein said *E. coli* strain J5 is of the Rc chemotype.

3. A vaccine of claim 1, wherein said *N. meningitidis* is group B strain.

5. A vaccine of claim 1, wherein the weight ratio of said purified outer membrane protein to said purified and detoxified endotoxin in said non-covalent complex is between 1 and 2.

6. A method of actively immunizing a subject against infection by heterologous Gram-negative bacteria and LPS endotoxin-induced pathology, comprising administering to said subject an effective amount of a vaccine according to claim 1.

7. A method of claim 6, wherein said *E. coli* strain J5 is of the Rc chemotype.

8. A method of claim 6, wherein said *N. meningitidis* is group B strain.

15. An immunogenic composition comprised of the vaccine according to claim 1 in a pharmaceutically effective carrier.

16. A composition of claim 15, wherein said *E. coli* strain J5 is of the Rc chemotype.

17. A composition of claim 15, wherein said *N. meningitidis* is group B strain.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 71007/137/USGO

In re patent application of
Apurba BHATTACHARJEE *et al.*
Serial No. 08/230,402
Filed: April 20, 1994
For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

Group Art Unit: 1802
Examiner: H. Sidberry

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Alan S. Cross, M.D., declare and say as follows:

1. I am the Alan S. Cross shown as coinventor on the captioned patent application.

2. I have had twenty three years of experience in the field of vaccines directed against bacterial infections. My *curriculum vita* is enclosed.

3. I have read in detail Examiner Paper No. 13, an Office Action in the captioned application mailed October 29, 1996 by Examiner H. Sidberry.

4. In my opinion, there is no basis for the Examiner's assertions that Zollinger *et al.*, United States Patent No. 4,707,543 anticipates or makes obvious the present invention; the differences are striking at every level, as I will outline below:

4.1. In lines 4-5 of the Zollinger Abstract, it is stated that the patent is to products useful as vaccines against infection ". . . by the same bacteria" . . . and protecting

In sharp contrast, our vaccine protects against heterologous infections. See, Example 10, p.20 and Example 11, p.22. This is now a limitation on all claims.

4.2 Zollinger is concerned with the interaction of vaccine components with the homologous bacteria from which the vaccines are derived.

4.3 Zollinger provides no evidence of cross-protection.

4.4 Zollinger provides no evidence of passive immunization, which is a property of the present vaccine.

4.5 Zollinger recites that the LPS portion of the vaccine can be substituted with the LPS of other Gram-negative bacteria, including *E. coli*. Zollinger implies that these LPSs could provide only type-specific protection. In other words, if the LPS were obtained from *E. coli* 018, then it would be effective only against infection with *E. coli* 018. No data is presented on this subject by Zollinger, and no discussion is provided. It would not be apparent from the Zollinger disclosure that the substitution of the meningococcal polysaccharide or LPS with that of *E. coli* would provide heterologous protection.

5. A second element of the claimed invention that is not anticipated or obviousness-making by Zollinger, is the present role of OMP strictly as an adjuvant. OMP induces no protective activity of its own. Rather, it maintains the LPS in a proper spatial configuration such that relevant cross-reactive epitopes are exposed in a manner different then when they simply are conjugated to protein given alone. Thus, the concepts of the protective antibodies are quite different between Zollinger and ourselves.

6. The present type of antibody induced is also different. In the Zollinger patent, what were produced were

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

Respectfully submitted,

3/2/97
Date

Alan S. Cross, M.D.

6/19/98

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 71007/137/USCO

In re patent application of
Apurba BHATTACHARJEE et al.
Serial No. 08/230,402
Filed: April 20, 1994
For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

Group Art Unit: 1641
Examiner: S. Loring

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Alan S. Cross, M.D., declare and say as follows:

1. I am the Alan S. Cross shown as coinventor on the captioned patent application.

2. I am experienced in the field of vaccines directed against bacterial infections. My curriculum vita is appended to my prior declaration.

3. Zollinger discloses vaccines comprising outer membrane protein and polysaccharide, in which the polysaccharide portion of the vaccine can be capsular polysaccharide or lipopolysaccharide (LPS) of any Gram-negative bacteria, with *E. coli* being just one of the possibilities. By contrast, the present claims recite combinations of outer membrane protein (OMP) derived from *N. meningitidis* and purified, detoxified LPS endotoxin derived from a particular mutant strain of *E. coli* that lacks O-polysaccharide sidechains, the J5 strain.

4. Combinations of OMP derived from *N. meningitidis* and purified, detoxified LPS endotoxin derived from *E. coli* strain J5 provide unexpectedly superior protection against gram-negative sepsis as compared to combinations of OMP with LPS purified, detoxified endotoxins from other strains of *E. coli*.

Serial No.: 08/886,044

In our studies, we have complexed OMP derived from *N. meningitidis* with other lipopolysaccharides, including LPS endotoxin derived from a strain of Brucella and from *E. coli* 018 (EC018). Ten mice/group were immunized with PBS or with 20 µg of vaccine (OMP, Brucella-OMP, J5-OMP, or EC018-OMP) at day 0, day 14 and day 28. EIA against all vaccine antigens, lipid A and Re LPS was done on sera drawn prior to challenge. Seven days after the immunization protocol was completed, the mice were challenged with 100 ng of EC018 lipopolysaccharide and 20 mg galactosamine intraperitoneally. Thus, challenge was homologous with respect to mice immunized with EC018-OMP, while challenge was heterologous with respect to mice immunized with J5-OMP.

5. There were no survivors among mice immunized only with OMP. No protection was provided by immunization with Brucella-OMP. Some protection was expected for mice immunized with EC018-OMP, since challenge was with the homologous strain. Sixty percent survival ($p=0.01$) was observed in the group of mice immunized with EC018-OMP. In mice immunized with J5-OMP, however, survival was 90% ($p=0.0001$), i.e., vaccination with J5-OMP provided 50% greater protection than vaccination with EC018-OMP. This was particularly surprising in view of the fact that J5-OMP vaccine was providing protection against infection by a heterologous strain (EC018) whereas EC018-OMP was providing protection against the same strain. LPS endotoxin from *E. coli* J5 in combination with OMP from *N. meningitidis* clearly provides protection that is markedly superior to LPS endotoxin from other strains of *E. coli* in combination with OMP from *N. meningitidis*.

6. A clinical protocol to test the safety of the present vaccine for immunising a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology has been written for Phase I trials in humans, and has been approved by (1) the Walter Reed Army Institute of Research (WRAIR)

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Scientific Review Committee; (2) the WRAIR Institutional Review Board (IRB); and (3) the Surgeon General's Human Subjects Research Review Board (pending only the formality of my being credentialed at WRAIR so that I may act as principal investigator). My co-inventor Dr. Bhattacharjee has consulted with Dr. Richman of the FDA about the specifics of the protocol, who suggested minor modifications to the Phase I trial. These modifications were incorporated, and the protocol will be submitted, along with the IND application, to the FDA.

I further declare that all statements made in this declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of any patent that may issue based on them.

Respectfully submitted,

June 19, 1998
Date

Alan S. Cross, M.D.

1/24/98

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 71007/137/USGO

In re patent application of

Apurba BHATTACHARJEE *et al.* Group Art Unit: 1641

Serial No.: 08/886,044 Examiner: S. Devi

Filed: June 20, 1997

For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

I, **ALAN S. CROSS, M.D.**, of 6810 Brookville Road, Chevy Chase, Maryland 20815, do solemnly and sincerely declare that:

1. I am one of the inventors of the inventions disclosed and claimed in the patent application captioned above. My *Curriculum Vitae* is attached to a prior declaration.

2. Attached as Exhibit 1 are Methods and Results relating to active immunization with a detoxified *Escherichia coli* J5 LPS-Group B meningococcal outer membrane protein complex vaccine, and subsequent challenge with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

3. Active immunization with J5 dLPS/OMP vaccine produced a prompt and sustained anti-core glycolipid antibody level that was generally in 100-fold excess of pre-immunization baseline levels. Twenty-four hours after onset of bacteremia, antibody levels decreased, but then rapidly recovered to, and remained at, pre-infection levels. Active immunization with J5 LPS/OMP vaccine induced greater than 800 ELISA units/ml of antibody at the onset of neutropenia, nearly 4 weeks after the last dose of vaccine, and this

level persisted throughout the entire period of neutropenia, for up to 80 days after the initial immunization. This is in distinct contrast to results achieved by passive immunization with antibodies, where initial levels of 800 ELISA units/ml of antibody dropped to less than 200 ELISA units/ml of antibody by 24 hours.

4. Immunization did not prevent either systemic infection or initiation of sepsis, but it did reduce the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Vaccinated animals challenged with *Pseudomonas aeruginosa* had an overall survival rate of 48% compared to 7% for saline treated control animals. A similar result ensued with *Klebsiella pneumoniae* challenge, with a 64% survival rate for vaccinated animals versus a 13% survival rate for control animals.

5. Vaccinated animals had the same frequency and magnitude of bacteremia from the challenge strain as the control group, but had significantly lower levels of bacteria in liver and spleen than control animals. I hypothesize that antibodies generated in response to the vaccine promote the uptake and killing of bacteria from the blood by tissue.

6. In addition to the decreased bacterial levels in liver and spleen, there was a significantly lower level of circulating endotoxin at the onset of fever in vaccinated animals as compared to control animals. While endotoxin levels increased in both groups at 24 hours, they were still lower than those of the control group. The lower level of circulating endotoxin may be due in part to promotion of LPS clearance from the circulation.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

1/12/99
Date

Alan S. Cross, M.D.

METHODS

Bacterial Challenge. Two bacterial pathogens were used in these experiments; *Pseudomonas aeruginosa* 12.4.4. and *Klebsiella pneumoniae* K2. *P. aeruginosa* 12.4.4. (originally provided by A. McManus; United States Army Institute of Surgical Research, San Antonio, TX) is a serum-resistant, human blood stream isolate of *P. aeruginosa*. The organism belongs to Fisher-Devlin-Gnabasik immunotype 6. The organism was stored in 10% glycerin at -70°C until ready for use. The day prior to the oral challenge, the isolate was incubated overnight in trypticase soy broth (TSB:Becton Dickinson, Cockeysville, MD) at 37°C . The following day bacteria were suspended in normal saline and adjusted spectrophotometrically to an inoculum size of 10^6 CFU/ml. This dose exceeds the LD_{90} for this experimental model in previous studies (6-8).

Klebsiella pneumoniae K2 (strain B5055) is a serum-resistant, rodent-virulent, encapsulated strain of *K. pneumoniae* (originally obtained from Drs. Frits and Ida Orskov, Staatsserum-Institut, Copenhagen Denmark). The organism was stored and prepared as described above with exception that the challenge dose was 10^7 CFU/ml since preliminary studies demonstrated that a higher inoculum was necessary to achieve a dose that exceeds the LD_{90} in this animal model.

Vaccine. The vaccine used in these experiments is a non-covalent vaccine consisting of detoxified *Escherichia coli* J5 lipopolysaccharide and *Neisseria meningitidis* group B outer membrane protein (7). When this de-O-acylated J5 lipopolysaccharide is complexed with the OMP from group B, *N. meningitidis*, it remains highly immunogenic and is a well-tolerated in experimental animals (7). The vaccine

was stored at 4°C until ready for use. The vaccine was administered at a dose of 20 μg subcutaneously at intervals of 0 and 4 weeks (two dose schedule) or 0, 2 and 4 weeks (3 dose schedule). The temperature was checked by infra-red thermometry (Horiba) 24 hr prior, 1, 2, 24 and 48 hr after each immunization. The weights were checked weekly.

Animal Model. The basic design of the neutropenic rat model has been described in detail previously (6-8). Briefly, the female, non-pregnant, specific pathogen-free, albino, Sprague-Dawley rats weighing between 125 and 150 gm (Charles River Breeding Labs, Wilmington, MA) were maintained in filtered, biological safety cages and allowed to eat and drink ad libitum. After a seven day control period, the animals underwent baseline blood sampling and then were immunized the J5 dLPS/OMP complex without the addition of an adjuvant. Two weeks after the last dose of the vaccine, repeat blood sampling was performed to determine vaccine responsiveness. Four weeks after the last dose of vaccine, animals were then rendered neutropenic with cyclophosphamide (Bristol-Meyers, Evansville, IN) at a dose of 100 mg/kg (time 0) IP followed by a second dose of 50 mg/kg IP 72 hours later to induce neutropenia.

Cefamandole (100 mg/kg) (Eli Lilly, Indianapolis, IN) was given IM beginning 96 hours before the first dose of cyclophosphamide to facilitate colonization of the alimentary tract with the challenge strain of *P. aeruginosa*. Ampicillin (Sigma, St. Louis, MO) was given at a dose of 25 mg/kg IM and orally on an every other day basis to disrupt colonization resistance against *Klebsiella pneumoniae*.

The activity of the vaccine was also tested in the presence of active antimicrobial therapy against the challenge strain of *P. aeruginosa*. At the onset of fever ceftazidime (50 mg/kg) (Glaxo Wellcome, Research Triangle Park, NC) was given IV in one

experimental group with (n=18) and without (n=10) the active vaccine. The treatment was given intravenously at a low dose (to promote antibiotic-induced endotoxin release [9]) every 12 hours for 48 hours after the onset of fever in these septic animals.

The bacterial challenge was given orally via orogastric tube prepared from polyethylene tubing (Intramedic PE, 160: Clay Adams Division, Becton Dickenson, Parsippany, NJ). The bacterial challenge was given on day 0 (the first dose of cyclophosphamide) and again on days 2 and 4. Phosphate buffered saline (PBS) was given as a control for the intravenous injections and for the vaccine placebo groups. A bacterial suspension was prepared to deliver 1 ml of 10^6 CFU *P. aeruginosa* 12.4.4 or 10^7 CFU *K. pneumoniae* K2 for each experimental group.

All manipulations were done under light CO_2 anesthesia to minimize any stress or trauma to the animals. Before onset of neutropenia, a patch of fur approximately 4x4 cm was shaved off the lateral thoracic region of the animal to allow for accurate and repeated body temperature recordings. A Horiba non-contact digital infrared thermometer (Markson Science - Phoenix, AZ) was used to monitor the animal's body temperature several times daily. Fever generally occurred in infected animals 4-5 days after the initial dose of cyclophosphamide; fever was defined as a body temperature measurement >38.0°C. The experiments were approved by the Brown University Animal Care Committee and were in accord with national guidelines for laboratory animal facilities and care.

Blood determinations and necropsy studies. Blood samples were obtained from the retro-orbital plexus of each animal under CO_2 anesthesia prior to immunization; two weeks after the four week immunization schedules; two days prior to the first dose of

cyclophosphamide; at the onset of fever; and 24 hours after the onset of fever. Each blood sample was tested for quantitative bacterial counts, serum endotoxin levels, and anti-J5 antibody levels. Quantitative bacteriology was performed using standard methods with serial dilutions of whole blood performed in TSB. The limit of detection was 10 CFU/ml of blood. Blood and tissue specimens from animals challenged with *P. aeruginosa* 12.4.4 were plated on Pseudomonas Isolation agar (Difco, Detroit, MI). Non-lactose-fermenting, oxidase-positive colonies were identified and immunotyped with polyvalent *P. aeruginosa* antisera (Difco, Detroit MI). In *K. pneumoniae* K2 challenge experiments, cultures were plated on Simmon's Citrate media (Becton Dickenson, Cockeysville, MD) and then characterized using standard microbiologic methods. The bacterial colony counts from the liver and spleen were measured separately for each animal but since the colony counts from the two sites were so similar, the results were combined and reported compositely as CFU/gm tissue.

Endotoxin levels were measured in serum samples which were heat-treated to 70°C after a 1:10 dilution in endotoxin-free water. Endotoxin measurements were determined by turbidimetric quantitative limulus amebocyte lysate assay (Associates of Cape Cod, Woods Hole, MA). Anti-J5 antibody titers were measured using an ELISA method previously described (6,7).

Each animal was examined daily throughout the experiment until 14 days after cyclophosphamide treatment. Previous experiments (6-8) have shown that the period of neutropenia (<50 granulocytes/mm^3) induced by this dose regimen of cyclophosphamide begins three days after the first dose of cyclophosphamide and extended until days 10-12. Animals that remain alive for >14 days after the cyclophosphamide treatment were

considered long-term survivors. All animals that succumbed during the course of the experiment were subjected to necropsy examination with quantitative cultures obtained from the cecum, liver, spleen, and lung tissue. Animals that survived the experiment were sacrificed and a necropsy examination with quantitative cultures of the same organ samples are obtained.

EndoCab assay. This assay for antibody to core glycolipids was performed as previously described (10,11). Briefly, 96 well microtiter plates were coated with a mixture of one rough LPS (incomplete outer core of chemotypes Rc or Rb) from *E. coli, Salmonella, Klebsiella* and *Pseudomonas aeruginosa.* Serum samples were added to the wells and anti-core glycolipid antibodies bound were detected by alkaline-phosphatase conjugated goat anti-rabbit IgG.

Data Analysis and Statistical Methods. Survival functions were measured using Kaplan-Meier plots and differences in survival time were measured using a non-parametric Kruskal-Wallis one-way analysis of variance. Numeric data was compared using the Mann-Whitney U-test. Numeric data are expressed as mean ± standard error; p values <0.05 was considered significant.

RESULTS

Vaccine Response. Both 2 dose and 3 dose vaccine schedules were studied in preliminary experiments. Animals vaccinated with the J5 dLPS/OMP complex vaccine (n=40) experienced no febrile reactions for up to 48 hrs after each immunization and had feeding and weekly weight gain patterns which were not different from the saline-

immunized control groups (n=31). The two vaccine schedules resulted in anti-J5 antibody levels which exceeded the target antibody response of 800 ELISA units (Figure 1). This level of antibody response was predicted to be protective based upon previous experiments with passively administered, rabbit-derived antisera (6). Since the 3 dose vaccine regimen resulted in significantly greater ($p<0.05$) antibody titers (2,440±526 ELISA units, n=40) than the 2 dose regimen (840±175 units, n=15), the 3 dose schedule was exclusively used in subsequent challenge experiments with *P. aeruginosa* and *K. pneumoniae*.

Vaccine effects on survival. Pseudomonas infection in the absence of ceftazidime. The circulating granulocyte levels were below 50 granulocytes/mm^3 in a sample of animals (n=10) tested 3 days after treatment with cyclophosphamide. Antibody elicited by this vaccine protected neutropenic rats from lethal *Pseudomonas* infection when passively infused as treatment at the onset of fever (7). We therefore examined whether this vaccine induced protection against lethal sepsis when actively administered as prophylaxis before the induction of neutropenia and infection. A Kaplan-Meier survival plot of vaccinated and control groups of neutropenic animals who received *Pseudomonas aeruginosa* 12.4.4. oral challenge is depicted in Figure 2. Vaccinated animals had an overall survival rate of 48% (13/28) while saline treated control animals had a survival rate of 7% (2/29) ($p<0.01$).

After the third dose of vaccine, there was a prompt (by day 35, 7 days following the last dose of vaccine) and sustained (>12 weeks) anti-core glycolipid antibody levels which were generally 100-fold in excess of pre-vaccine baseline levels (Table 1, see

below). Antibody titers diminished slightly over the course of bacteremic infection in *Pseudomonas aeruginosa*- challenged animals (Table 1). Twenty-four hr after infection anti-J5 LPS antibody levels decreased, but then rapidly recovered to pre-infection levels and remained elevated throughout the duration of the experiment (3 months). The saline-treated control animals had anti-J5 antibody levels which were at the limits of detection throughout the experimental period (3 months).

Circulating levels of bacterial endotoxin were undetectable or very low prior to the onset of infection in vaccinated and control animals challenged with *P. aeruginosa* in the absence of ceftazidime therapy (Figure 2). Vaccinated animals had a significantly lower level of endotoxin at the onset of fever during the course of *P. aeruginosa* infection in these immunocompromised animals. However, endotoxin levels were elevated to a similar degree in vaccinated and control groups after 24 hours of continued fever and overt illness in these neutropenic animals (Figure 2).

Pseudomonas infection in the presence of ceftazidime. Since antibiotic treatment may liberate endotoxin from the dying bacteria (9), we tested the ability of actively-induced antibody to protect animals from lethal sepsis under conditions in which there may be an acute endotoxin load. A similar level of protection was observed in animals who received vaccine and ceftazidime at the onset of fever (Figure 4) as was observed in animals receiving vaccine alone (Figure 3) (i.e. approximately 60 % survival). Ceftazidime was highly active *in vitro* against this strain of *P. aeruginosa* 12.4.4 (MIC=0.25 μg/ml). Ceftazidime-treated animals cleared the Pseudomonas bacteremia (0 cases of bacteremia/10 animals) after 24 hr of therapy, yet this dose of ceftazidime, while prolonging survival compared to animals not receiving antibiotics, was unable ultimately

to protect these neutropenic animals from lethality (Figure 4). In contrast, the J5 dLPS/OMP vaccine significantly improved mortality (11/18 survived, $p<0.01$) in combination with ceftazidime (Figure 4).

At the onset of fever and 24 hr later, endotoxin levels remained significantly elevated in non-vaccinated animals treated with ceftazidime ($n=10$; 5.45±2.2 ng/ml) and these circulating endotoxin levels were not significantly different from the saline-immunized control group ($n=4$; 7.7±3.3 ng/ml) (p=NS). Ceftazidime-treated animals who also received the J5 dLPS/OMP vaccine, however, had the lowest endotoxin levels within the first 24 hr after fever onset ($n=18$; 2.9±1.5 ng/ml) ($p<0.05$, compared to non-immunized animals).

Klebsiella infection in the absence of ceftazdime. Previous studies in this neutropenic rat model used *Pseudomonas aeruginosa* as the primary challenge strain. If this J5 dLPS/OMP vaccine is to have broad clinical applicability in the prevention and/or treatment of gram-negative bacterial sepsis, it should be efficacious against infections caused by other heterologous gram-negative bacilli. *K. pneumoniae* challenge was highly lethal in the saline control group (Figure 5). The Kaplan-Meier survival plots of animals that received the J5 dLPS/OMP complex vaccine ($n=14$) and the control group ($n=15$) is depicted in Figure 5. The vaccine provided a highly significant survival protection in these neutropenic animals (9/14 of immunized animals survived v. 2/15 of saline-immunized) ($p<0.005$). As was observed in animals infected with Pseudomonas, there was a decrease in anti-J5 LPS antibody levels at 24 hr after onset of fever in Klebsiella-infected animals, but here, too, the levels returned to pre-febrile levels (data not shown).

Thus active immunization with the J5 dLPS/OMP vaccine provided a survival advantage for infection with both *Pseudomonas* and *Klebsiella* species.

Endotoxin levels in the circulation of animals infected with *Klebsiella pneumoniae* K2 were significantly reduced in the vaccine-treated group. Blood levels of endotoxin 24 hr after the onset of fever were 0.75±.43 ng/ml in the vaccinated animals while the endotoxin levels were 4.9±1.5 ng/ml in the control group ($p<0.01$).

Bacterial load. Multisystem infection with either *P. aeruginosa* 12.4.4 or *K. pneumoniae* K2 occurred invariably in the control group, resulting in a >90% mortality (Figures 3-5). In each experiment, vaccinated animals had the same frequency and magnitude of bacteremia from the challenge strain as the control group. When measured within the first 24 hr after fever onset, the quantitative level of bacteremia in *P. aeruginosa*-challenged animals was 76±23 CFU/ml (vaccine group) and 205±150 CFU/ml (control group) (p=NS). The quantitative level of bacteremia following *K. pneumoniae* challenge was 583±280 CFU/ml (vaccine group) vs. 412±201 CFU/ml (control group) (p=NS).

Despite no significant differences in circulating levels of bacteremia, quantitative bacterial counts of organ tissue cultures revealed that vaccinated animals had significantly lower tissue levels of the challenge organism when compared to the control groups in both the *Pseudomonas* and *Klebsiella*-challenged animals. In the animals challenged with *P. aeruginosa* in the absence of ceftazidime, the tissue levels in the vaccine group (n=28) were 401±177 CFU/mg tissue, while the control group (n=29) had tissue levels of 2,342±693 CFU/mg ($p<0.01$). In ceftazidime-treated animals challenged

with *P. aeruginosa* the organ bacterial colony count was 571±352 CFU/mg for those receiving both vaccine and antibiotic (n=18), 2,789 ± 1,726 CFU/mg in those receiving antibiotic alone (n=10) and 2,665 ± 1,994 CFU/mg in animals receiving neither vaccine nor antibiotic (n=4) ($p<0.01$). The quantitative tissue levels in *K. pneumoniae* K2-challenged animals in the vaccine group (n=14) was 127±101 CFU/mg while the levels in the control group (n=15) was 3,683±224 CFU/mg ($p<0.001$).

EndoCab assay. In separate experiments the sera from rabbits immunized with two doses of this J5 dLPS/OMP vaccine was tested for its ability to bind to a mixture of Rc or Rb chemotype LPS (10,11). Unlike normal rabbit IgG, the vaccine-immune sera had easily detectable titers of antibody to the LPS mixture, as did the sera from rabbits immunized with a mixture of core LPS antigens. Thus, the J5 dLPS/OMP vaccine induced a population of antibodies that bound to a similar panel of antigens to which serum from an unrelated vaccine composed of core LPS antigens bound.

References

1. Perl TM, Dvorak L, Hwant T, Wenzel RP. Long-term survival and function after suspected Gram-negative sepsis. JAMA 274:338-345, 1995.

2. Abraham E, Wunderink R, Silverman H, Perl TM, Nasraway S, Levy H, Bone R, Wenzel RP, Balk R, Alfred R, Pennington JE, Wherry JC. Efficacy and safety of monoclonal antibody to human tumor necrosis factor α in patients with sepsis syndrome. JAMA 273:934-941, 1995.

3. Fisher Jr CJ, Dhainaut JA, Opal SM, Pribble JP, Balk RA, Slotman GJ Iberti TJ, Rackow EC, Shapiro MJ, Greenman RL, Reines HD, Shelly MP, Thompson BW, LaBrecque JF, Catalano MA, Knaus WA, Sadoff JC. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. JAMA 271:1836-1878, 1994.

4. Ziegler EJ, Fisher CJ Jr, Sprung CL, Straube RC, Sadoff JC, Foulke GEG, Wortel CH, Fink MP, Dellinger RP, Teng NNH, Allen IE, Berger HJ Knatterud GL, LoBuglio AF, Smith CR, and the HA-1A Sepsis Study Group. Treatment of Gram-negative bacteremia in septic shock with HA-1A human monoclonal antibody against endotoxin: a randomized, double-blind, placebo-controlled trial. N Engl J Med 325:429-436, 1991.

5. Ziegler EJ, McCutchan JA, Fierer J, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant Escherichia coli. N Engl J Med 307:1225-30, 1982.

6. Bhattacharjee AK, Opal SM, Palardy JE, Drabick JJ, Collins H, Taylor R, Cotton A and Cross AS. Affinity-purified Eschereichia coli J5 Lipopolysaccharide-specific IgG protects neutropenic rats against gram-negative bacterial sepsis. J Infect Dis 170:622-629, 1994.

7. Bhattacharjee AK, Opal SM, Taylor R, Naso R, Semenuk M, Zollinger WD, Moran EE, Young L, Hammack C, Sadoff JC and Cross AS. A noncovalent complex vaccine prepared with detoxified Escherichia coli J5 (Rc Chemotype) Lipopolysaccharide and Neisseria meningitidis Group B outer membrane protein produces protective antibodies against gram-negative bacteremia. J Infect Dis 173:1157-1163, 1996

8. Collins HH, Cross AS, Dobek A, Opal SM, McClain JB, Sadoff JC. Oral ciprofloxacin and anti-lipopolysaccharide monoclonal antibody protect leukopenic rats from lethla infection. J Infect Dis 159:1073-82, 1989.

9. Jackson JJ, Kropp H. β-lactam antibiotic-induced release of free endotoxin: in vivo comparison of penicillin-binding protein (PBP) 2-specific imipenem and PBP-specific ceftazidime. J. Infect Dis 65: 1033-41, 1992.

10. Goldie AS, Fearon KCH, Ross JA, Barclay R, Jackson RE, Grant IS, Ramsay G, Blyth AS, Howie JC,. Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome. JAMA 1995;274:172-7.

11. Bennett-Guerrero E, Ayuso L, Hamilton-Daview C, White WD, Barclay R, Smith PK, King SA, Muhlbaier LH, Newman MF, Mythen MG. Relationship of preoperative antiendotoxin core antibodies and adverse outcomes folowing cardiac surgery. JAMA 1997;277:646-50.

12. Baumgartner JD, Glauser MP, McCutchan JA, et al. Prevention of gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. Lancet 2:59-63, 1985.

13. Calandra T, Glauser MP, Schellekens J, Verhoef J, Swiss-Dutch J5 Immunoglobulin Study Group. Treatment of gram-negative septic shock with human IgG antibody to Escherichia coli J5: a prospective, double-blind, randomized trial. J Infect Dis 158:312-9, 1988.

14. The Intravenous Immunoglobulin Collaborative Study Group. Prophylactic intravenous administration of standard immune globulin as compared with core-lipopolysaccharide immune globulin in patients at high risk of postsurgical infection. N Engl J Med 327:230-40, 1992.

15. Powderly WG, Pier G, Markham RB. *In vitro* T cell-mediated killing of Pseudomonas aeruginosa. IV. Nonresponsiveness of polysaccharide-immunized BALB/c mice is attributable to vinblastine-sensitive suppressor T cells. J. Immunol.1986;137:2025

16. Cross AS, Siegel G, Byrne WR, Trautmann M, Finbloom DS. Intravenous immune globulin impairs antibacterial defenses of a cyclophosphamide-treated host. Clin Exper Immunol 1989;76:159-

17. Ahlgren T, Berghem L, Grundfelt M-B, Dommellof L. 1980. Influence of cancer chemotherapy treatment on the reticuloendothelial system in the rat. Exper. Chemother 1980;26:128-34.

18. Ehrke MJ, Mihich E, Berd D, Mastrangelo MJ. Effects of anticancer drugs on the immune system in humans. Semin in Oncol. 1989;16:230-53.

19.MacLean GD, Miles DW, Rubers RD, Reddish MA, Longenecker BM. Enhancing the effect of THERATROPE St_n–KLH cancer vaccine in patients with metastatic breast

cancer by pretreatment with low dose cyclophosphamide. J Immunother with Emphasis on Tumor Immunol 19: 309-16, 1996.

20.Ertel W, Faist E, Nestle C. Kinetics of interleukin-2 and interleukin-6 synthesis following major mechanical trauma. J Surg res 48: 622-8, 1990.

21. Campbell WN, Hendrix E, Cryz SJ, Cross AS. Immunogenicity of a 24-valent Klebsiella capsular polysaccharide vaccine and an 8-valent Pseudomnas O-polysaccharide conjugate vaccine administered to acute trauma victims. Clin Infect Dis 1996;23:179-81.

22. Pollack M, Huang AI, Prescott RK, et al. Enhanced survival in Pseudomonas aeruginosa septicemia associated with high levels of circulating antibody to Escherichia coli endotoxin core. J Clin Invest 72:1874-81, 1983.

23. Schedel I, Dreikhausen U, Nentwig B, Hockenschneider M, Rauthmann D, Balikcioglu S, Coldewey R, Deicher H. Treatment of gram-negative septic shock with an immunoglobulin preparation: a prospective, randomized clinical trial. Crit Care Med 9:1104-1113, 1991.

24. Nys M, Damas P, Joassin L, Lamy M. Sequential anti-core glycolipid immunoglobulin antibody activities in patients with and without septic shock and their relation to outcome. Ann Surg. 217:300-306, 1993.

25. Baumgartner JD, Heumann D, Calandra T, Glauser MP. Antibodies to lipopolysaccharide after immunization of humans with the rough mutant Escherichia coli. J Infect Dis 163:769-72, 1991.

26. DeMaria A Jr, Johns MA, Berberich H, McCabe W. Immunization with rough mutants of *Salmonella minnesota*: initial studies in human subjects. J. Infect. Dis. 158:301-11, 1988.

27. Brun-Buisson C, Doyon F, Carlet J, et al. Bacteremia and severe sepsis in adults: A multicenter prospective survey in ICUs and wards of 24 hospitals. Am. J. Respir. Crit. Care Med 154:617-24, 1996

28. Vincent J-L, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin M-H, Wolff M, Spencer RC, Hemmer M, for the EPIC International Advisory Committee. The prevalence of nosocomial infection in intensive care units in Europe. JAMA 1995;274:639-44.

29. Quartin AA, Scein RMH, Kett DH, Peduzzi PN, DVA Systemic Sepsis Cooperative Studies Group. Magnitude and duration of the effect of sepsis on survival. JAMA 1997;277:1058-63.

30. Bennett IL Jr. Approaches to the mechanisms of endotoxin action. In:Braun W, Landy M, eds. Bacterial Endotoxins: Proceedings of a Symposium Held at the Institute of Microbiology of Rutgers, the State University,. New Brunswick: Institute of Microbiology, Rutgers, the State University; 196a: xiii-vi.

31. Gathiram P., Wells MT, Brock-Utne JG, Gafin SL. Antilipopolysaccharide improves survival in primates subjected to heat stroke. Circulat Shock 23:157-64, 1987.

32. Cohen J, Moore RH, Al Hashimi S, Jones L, Apperley JF, Aber VR. Antibody titers to a rough-mutant strain of Escherichia coli in patients undergoing allogeneic bone marrow transplantation. Evidence of a protective effect against graft-versus-host disease. Lancet 1987 1:8-11.

33. Brock-Utne JG, Gaffin SL, Wells MT, Gathiram P, Sohar E, James MF, Morrell DF, Norman RJ. Endotoxemia in exhausted runners after a long-distance race. S. Afric. Med J. 73: 533-6, 1988.

33. Baker B, Gaffin SL, Wells M, Wessels BC, Brock-Utne JG. Endotoxemia in racehorses following exertion. J So Afr Vet Assoc. 59:63-6, 1988.

Legends

Figure 1. **Antibody levels to J5 LPS following 2- and 3-dose immunization regimens.** Rats were immunized with the J5 dLPS/OMP vaccine given subcutaneously (20 μg/dose) at time 0 and 4 weeks (2 dose) and at time 0, and at 2 and 4 weeks (3-dose). Control rats were immunized with saline. Serum was harvested at 6 weeks and antibody levels determined by ELISA (see Methods). While both immunization regimens induced antibody above a previously determined protective level (2), the antibody level induced by the 3 dose regimen was significantly greater that that induced following 2 doses ($p<0.0.05$).

Figure 2. **Circulating serum endotoxin levels in J5 dLPS/OMP vaccine- and saline-immunized rats.** Rats were immunized at time 0, and at 2 and 4 weeks. At 14 days following the last vaccine dose, animals were given the first dose of cyclophosphamide and of *Pseudomonas*. Animals were then followed every 12 hours for the onset of fever. Serum drawn at the onset of fever (typically days 5-6 after the first dose of cyclophosphamide) and at 24 hr later, were evaluated for endotoxin levels by a *Limulus* amebocyte assay.

Figure 3. **Effect of J5 dLPS immunization on survival following challenge with *Pseudomonas*.** Animals were immunized with either saline or J5 dLPS/OMP vaccine at time 0, and at 2 and 4 weeks. Fourteen days after the last immunization, animals were given the first doses of cyclophosphamide and of *Pseudomonas*, and followed for the

onset of fever. A Kaplan-Meier survival curve was plotted. Two of 29 animals in the control group survived, whereas (13/28) of immunized rats survived ($p<0.01$) to the conclusion of the experiment, when the neutropenia resolved.

Figure 4. **Effect of J5 dLPS immunization on survival of rats infected with *Pseudomonas* and given 4 doses of ceftazidime every 12 hr at the onset of fever.** Rats were immunized and treated with cyclophosphamide and given bacteria as described in Figure 3. At the onset of fever, however, ceftazidime, which binds to penicillin binding protein 3, was given to induce the release of endotoxin from the bacteria. Rats that received neither vaccine nor antibiotic all died by days 6 and 10 respectively. There was an increase in survival (11/18) among animals that were immunized with J5 dLPS/OMP vaccine and given ceftazidime .

Figure 5. **Increased survival among rats immunized with J5 dLPS/OMP vaccine and challenged with *Klebsiella*.** Rats were immunized with 3 doses of vaccine as described earlier (Figures 2 and 3). Fourteen days after the last dose of vaccine animals were given the first dose of cyclophosphamide and of *Klebsiella* by gavage. Instead of cefamandole, animals were treated with ampicillin to overcome colonization resistance. Fever and survival was followed for 12 days. Increased survival was observed among rats actively immunized with the J5 dLPS vaccine.

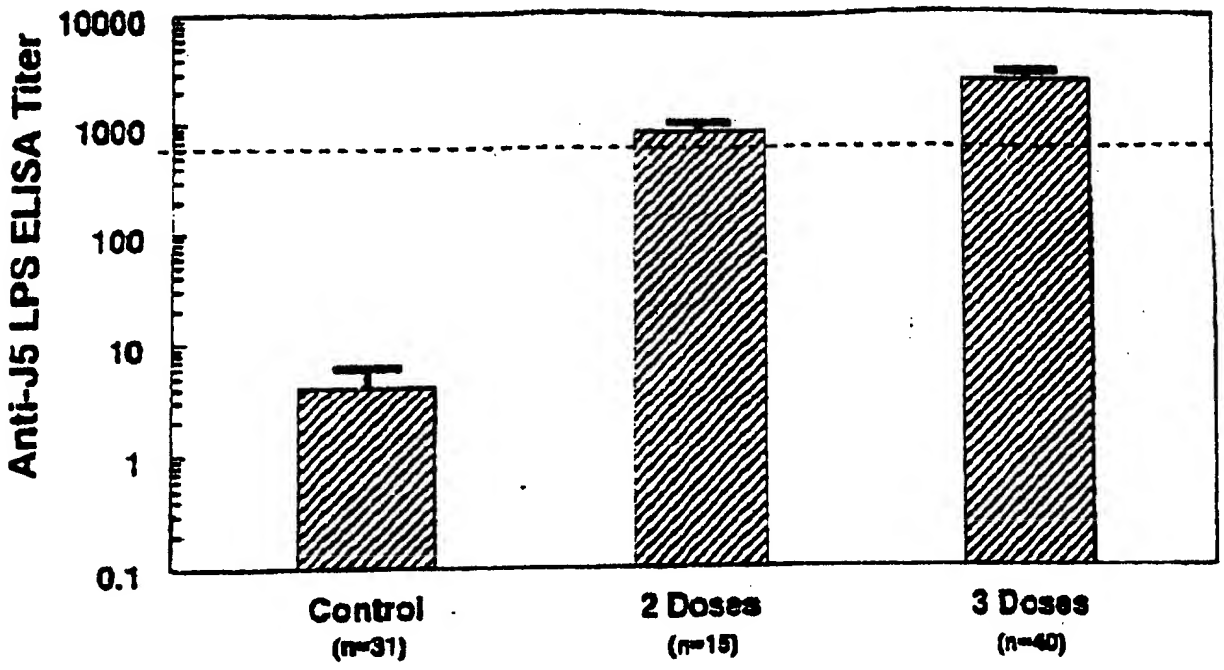
Figure 6. **Immunization with J5 dLPS elicited antibodies that reacted with a mixture of core glycolipid antigens from heterologous gram-negative bacteria.**

Antibody levels to a mixture of 4 different Rc or Rb LPS (one each from *E. coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Klebsiella*) each complexed to polymixin B. The sera from rabbits (130, 131, 134) immunized with a mixture of core antigens at monthly intervals x6 before being bled were compared to sera from two rabbits (anti-J5 dLPS, lots #1 and 2) immunized at time 0 and day 28 with the J5 dLPS/OMP vaccine. The level of antibody to core LPS structures in normal rabbit IgG (Sigma, St. Louis, MO) is shown for comparison.

TABLE 1. E. COLI J5 ANTIBODY LEVELS (ELISA UNITS)

TREATMENT	PRE-VACCINE (day 0)	POST-VACCINE (day 35)	ONSET OF SEPSIS	24 HR. POST ONSET OF SEPSIS	RECOVERY* (14 days after sepsis)
Vaccine (n=28)	6.4 ± 2.0	2852 ± 191**	2467 ± 527**	1827 ± 488**	2690 ± 663**
Control (n=29)***	7.5 ± 1.9	5.7 ± 3.0	8.5 + 2.8	7.6 ± 2.8	7.9 ± 3.1

Animals were immunized subcutaneously at days 0, 14 and 28 with 20 μg of the J5 dLPS/OMP vaccine or with normal saline. IgG antibody levels to J5 LPS were measured 7 days after the third dose. Thirty-three days after the third dose (61 days after the first dose of vaccine) animals were given the first dose of cyclophosphamide and *Pseudomonas aeruginosa* (see Methods). Additional antibody levels were measured at onset of sepsis (day 66), 24 hours later (day 67) and in surviving animals, at recovery (day 80). *Data from the 2 long term survivors from control group and from 13 long term survivors in the vaccine group; **$p<0.0001$ compared to pre-vaccine levels; ***p=ns all post-vaccine time points compared to pre-vaccine levels.



J5 dLPS/OMP given SQ (20ug/dose) 0,4wks-2 dose; 0,2,4wks-3 dose

Figure 1

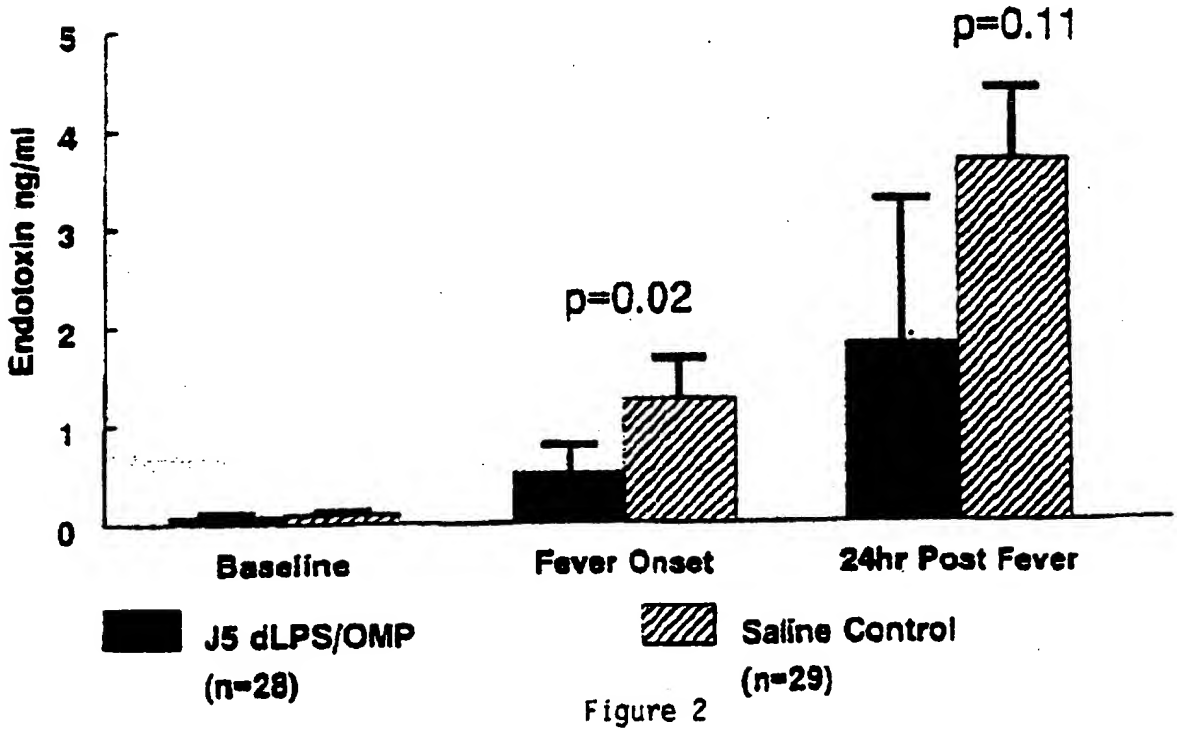


Figure 2

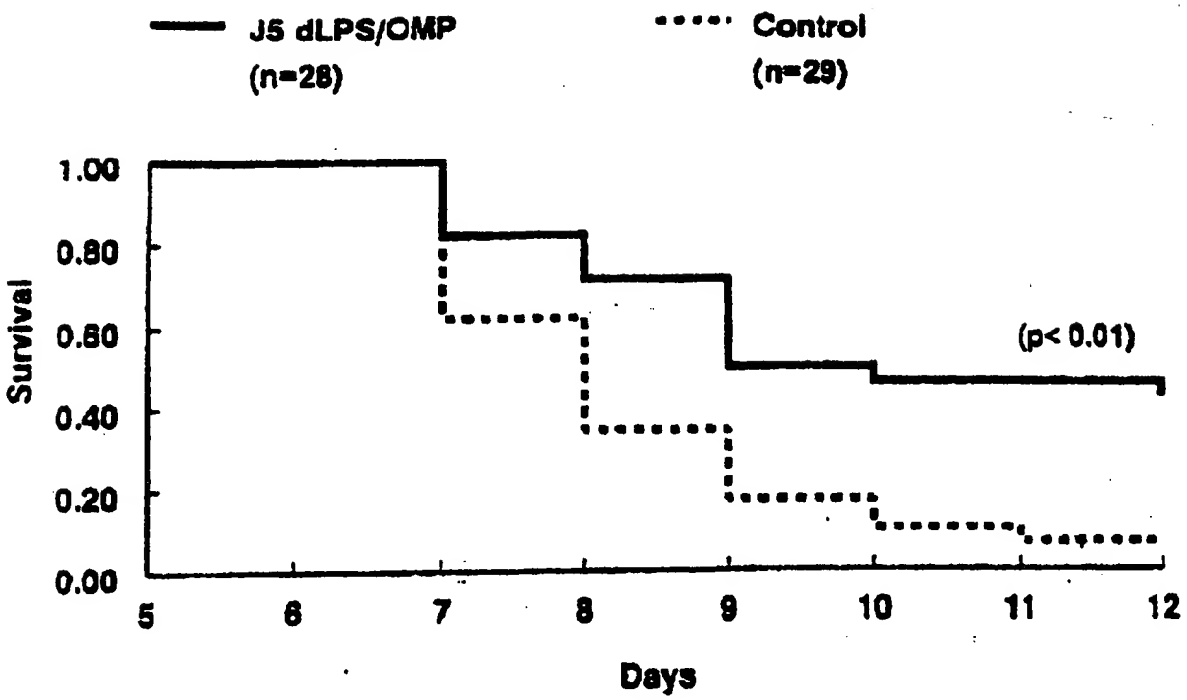


Figure 3

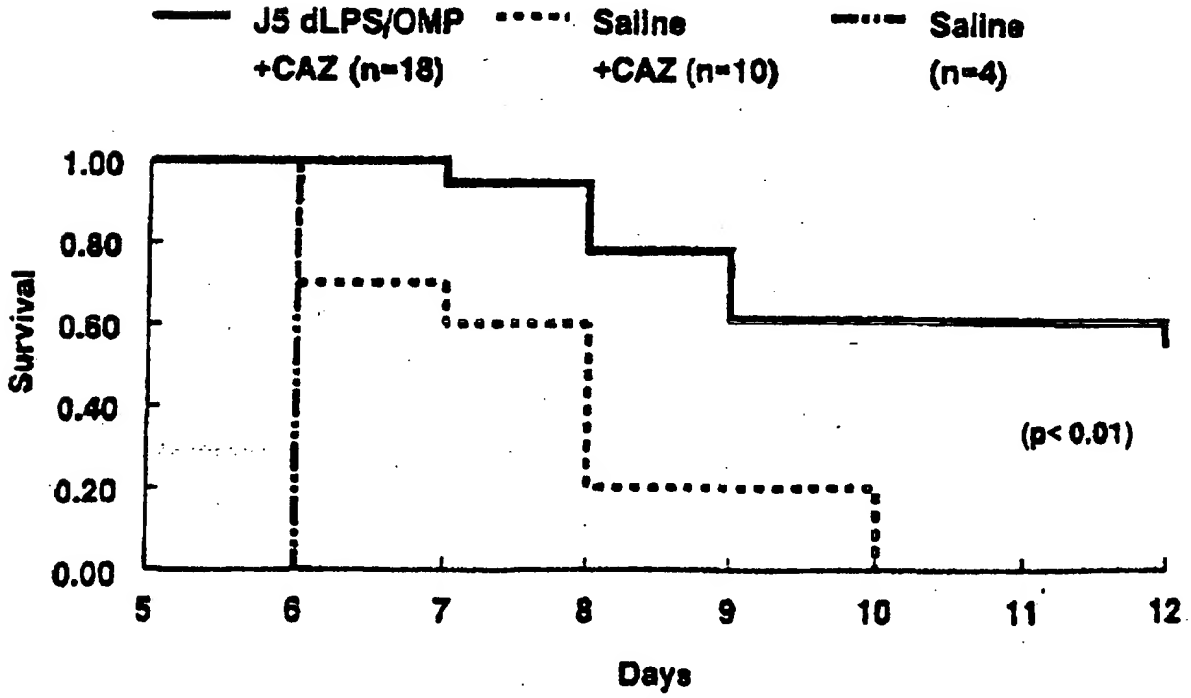


Figure 4

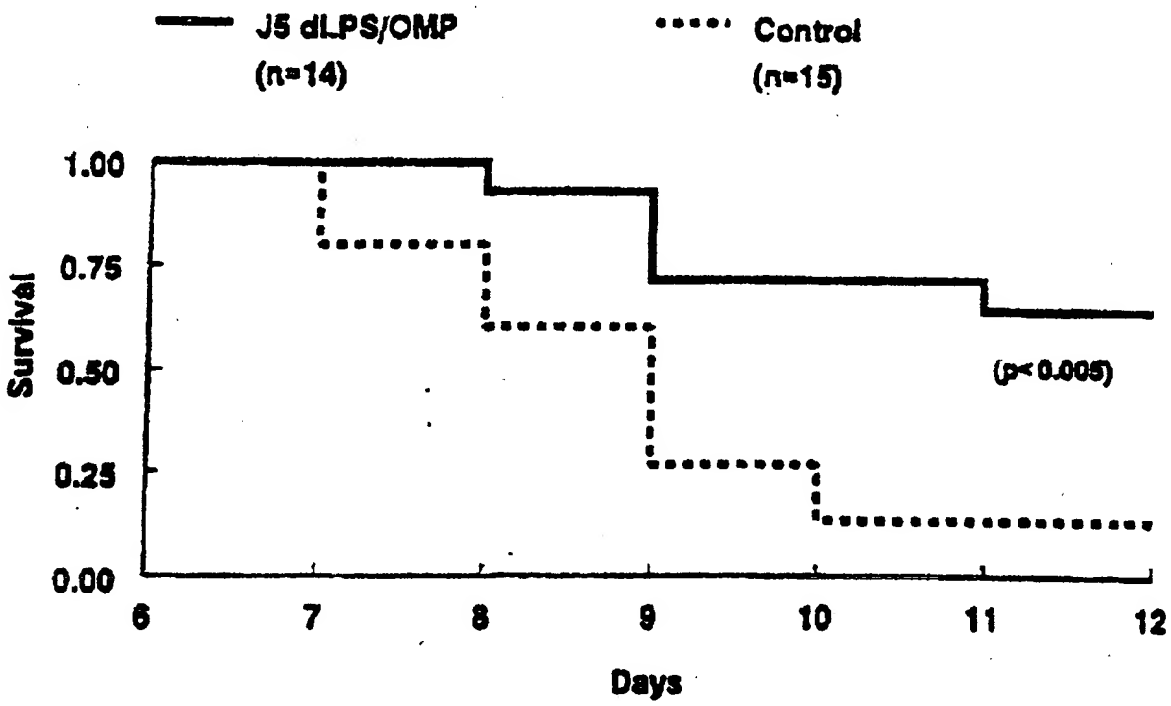

J5 dLPS/OMP
(n=14)
Control
(n=15)
1.00
0.75
0.50
0.25
0.00
Survival
(p<0.005)
6
7
8
9
10
11
12
Days

Figure 5